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December 14, 2006

Laura Tarantino, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: Medium- and Long-Chain Triacylglycerol (MLCT)-Oil GRAS Notification

Dear Dr. Tarantino:

In accordance with proposed 21 CFR § 170.36 (a notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the representative of the notifier, Nisshin OilliO Group USA, Inc., 120 Charlotte Place, Mid Level, Englewood Cliffs, NJ 07632, a GRAS notification for Medium-and-Long-Chain Triacylglycerol (MLCT)-Oil for use as a food ingredient, such that the total daily consumption of MLCT-Oil would not exceed 31 g/day. A GRAS expert panel dossier, setting forth the basis for the GRAS determination, as well as CVs of the members of the GRAS panel, is enclosed.

Best regards,

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR § 170.36 (c) (1)

Medium- and long-chain triacylglycerol (MLCT)-Oil has been determined to be generally recognized as safe (GRAS) and; therefore, exempt from the requirement of premarket approval, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

13 Dec 06

Date

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
888 Seventeenth Street, NW, Suite 810
Washington, DC 20006

(i) Name and Address of the Notifier

Katsuaki Yamanouchi
The Nisshin OilliO Group USA, Inc.
120 Charlotte Place, Mid Level
Englewood Cliffs, NJ 07632

Agent of Notifier:

George A. Burdock, Ph.D.
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Burdock Group
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(ii) Common Name of the Notified Substance

The common name of medium- and long-chain triacylglycerol (MLCT)-Oil has been defined as:

MLCT-Oil

(iii) Conditions of Use

MLCT-Oil may be used as a supplementary source of vegetable oil used in cooking, salad dressings, margarine spreads, and frozen dinners, provided that food standards of identity do not preclude such use.[1]

(iv) Basis of GRAS Determination

Pursuant to 21 CFR § 170.3, medium- and long-chain triacylglycerol (MLCT)-Oil has been determined GRAS by scientific procedures for its intended conditions of use. The safety of MLCT-Oil is supported by preclinical and clinical studies, and the fact that medium-chain triacylglycerols have been administered to patients with malabsorption syndromes, added to infant formulations, and consumed in the diet from natural sources, while long-chain

[1] Title 21 of the US Code of Federal Regulations (CFR), section 170.10, 2006

triacylglycerols contained in vegetable oils have been commonly consumed in the US diet. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food.

(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for the Food and Drug Administration's (FDA) review and copying at a reasonable time at the office of:

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
Burdock Group
888 Seventeenth Street, NW, Suite 810
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Alternatively, copies of data and information can be provided to FDA upon request, by contacting Dr. Burdock.

2. Detailed Information About the Identity of the Notified Substance

A. Identity

Medium- and long-chain triacylglycerol (MLCT)-Oil is an edible vegetable oil manufactured from common edible vegetable oils containing long-chain fatty acids, such as those found in rapeseed, soybean, cottonseed, sunflower seed, peanut, olive, corn, safflower, rice bran, and sesame seed oils, while medium-chain triacylglycerol (MCT) is produced from coconut and palm kernel oils. Specific ratios of edible vegetable oil and MCT produced from edible oils are combined with a food grade lipase utilized to promote a randomized ester exchange, such that the MLCT-Oil produced is composed of both medium- and long-chain triacylglycerols (MLCT). The medium-chain fatty acids (MCFA) of MLCT-Oil consist of only capric and caprylic fatty acids, each containing eight and ten carbons in length, respectively.

MLCT-Oil is produced in accordance with the manufacturing control standards and the quality control standards of the International Organization for Standardization (ISO) as a food grade ingredient, for use as an edible cooking oil, and used in salad dressings, margarine spreads, and frozen dinners, for which standards of identity do not preclude such use (Table 1).

Table 1. General description of medium- and long-chain triacylglycerol (MLCT)-Oil

Characteristic	Value
Appearance	Clear (Liquid oil equivalent in appearance to edible vegetable oils)
Packaging	Sealed in various packaging containers, including ethylene-vinylalcohol copolymer/polyethylene bags, aluminum bottles, tin cans, polyethylene terephthalate (PET) bottles, nylon/polyethylene (PE) pouches, or steel cans
Storage	Store at 20-25°C
Labeling	MLCT-Oil
Functional Use in Food	Source of vegetable oil fatty acids

Common or Usual Name:

The common name of medium- and long-chain triacylglycerol (MLCT)-Oil has been defined as:

MLCT-Oil

B. Composition

The chemical composition of MLCT-Oil is summarized in Table 2. MLCT-Oil is composed of a glycerol backbone with randomly bound medium and long chain fatty acids. Because medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA) are randomly attached *via* a food-grade lipase, MLCT-Oil medium (M) and long (L) chain triacylglycerol (MLCT) can have up to six possible configurations, *i.e.*, (1) L-L-L, (2) L-L-M, (3) L-M-L, (4) L-M-M, (5) M-L-M, and (6) M-M-M (Table 2). The fatty acids are derived from common edible oils rich in free medium and long chain fatty acids. Compositional analysis indicated that the fatty acids present in MLCT-Oil are the type commonly found in other edible oils (Table 3). The method of manufacture of MLCT-Oil is indicated in Figure 1.

Table 2. Triacylglycerol composition of MLCT-Oil

Fatty Acid	*Percent* (%)
L-L-L	49.5-52.7
L-L-M or L-M-L	37.3-39.6
L-M-M or M-L-M	8.6-9.3
M-M-M	0.1-0.2

M = medium-chain fatty acid; L = long-chain fatty acid; MLCT = medium- and long-chain triacylglycerol

Table 3. Fatty acid composition of MLCT-Oil

Fatty Acid	M/L	*Percent* (%)*	Fatty Acid	M/L	*Percent* (%)
Caprylic acid (C8:0)	M	8.5-9.1	Linoleic acid (C18:2)	L	16.1-18.8
Capric acid (C10:0)	M	2.7-2.8	Linolenic acid (C18:3)	L	5.4-10.3
Lauric acid (C12:0)	L	ND	Arachidic acid (C20:0)	L	0.4-0.6
Myristic acid (C14:0)	L	ND	Gadoleic acid (C20:1)	L	0.9-1.2
Palmitic acid (C16:0)	L	3.2-4.0	Behenic acid (C22:0)	L	0.2-0.4
Palmitoleic acid (C16:1)	L	0.1-0.2	Erucic acid (C22:1)	L	0.1-0.3
Stearic acid (C18:0)	L	1.6-1.8	Lignoceric acid (C24:0)	L	0.1-0.2
Oleic acid (C18:1)	L	49.0-54.2	Nervonic acid (C24:1)	L	0.1-0.3

*As a *percent* of the total fatty acid content; M= medium-chain fatty acid; L= long-chain fatty acid; ND= not detected; MLCT= medium- and long-chain triacylglycerol

C. Method of Manufacture of Medium- and Long-chain Triacylglycerol Oil

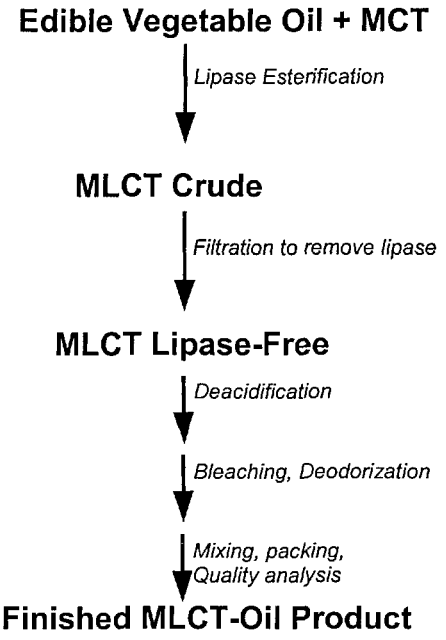

Figure 1. Medium- and long-chain triacylglycerol Oil (MLCT-Oil) production scheme

D. Specifications for Food Grade MLCT-Oil

Table 4. Specifications of medium- and long-chain triacylglycerol (MLCT)-Oil

			Batch Analysis Results (n = 5)	
Analysis	**Method**	**Specification**	**Range**	**Average**
Acid value	JOCS Official Methods 2.3.1. - 1996	Less than 0.15%	0.03 – 0.04%	0.04%
Appearance		Clear (Liquid oil equivalent in appearance to edible vegetable oils)	NA	NA
Arsenic	Flameless atomic absorption[a]	Less than or equal to 0.1 ppm	Passes	Passes
Color	JOCS Official Methods 2.2.1.1. - 1996	10Y/1.0R and below (133.4 mm cell)	2.6 – 6.0Y/0.2 – 0.6R	Passes
Heavy metals	Colorimetric method[a]	Less than or equal to 0.1 ppm	ND	ND
Iodine value	JOCS Official Method 2.3.4.1. - 1996	85-118	102.2 – 103.8	103.3
Peroxide value	JOCS Official Method 2.5.2.1. - 2003	Less than 10 meq/kg	0.0-0.6 meq/kg	0.3 meq/kg
Refractive Index	JOCS Official Method 2.2.3. - 1996	1.4665-1.4715	1.4686 – 1.4688	1.4687
Unsaponification value	JOCS Official Method 2.4.8. - 1996	Not more than 1.5%	0.84 – 1.02	0.91
Soap	AOCS Official Method Cc17-95	Less than or equal to 5.0 ppm	0.0	0.0
Moisture	JOCS Official Method 2.1.3.4. - 1996	Less than or equal to 0.2%	0 – 0.02%	0.01%
Medium chain fatty acid content (%)	D-46[a]	11.1 – 12.5 g/100 g	11.8 – 12.3%	12.1%

[a]Nisshin Method. SOP available on request; AOCS = American Oil Chemists' Society; JOCS = Japan Oil Chemists' Society; MLCT = medium- and long-chain triacylglycerol; NA = Not appropriate; ND = Not detected; ppm = parts *per* million; R = Red; Y = Yellow

3. Self Limiting Levels of Use

The quantity of MLCT-Oil use is not considered self-limiting,[2] any more than conventional edible vegetable oil.

4. Basis of GRAS Determination

The determination that MLCT-Oil is GRAS is on the basis of scientific procedures. See attached- DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT. On the basis of the data and information described in the attached dossier and other publicly available information, there is consensus, among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that MLCT-Oil is generally recognized as safe under the intended conditions of use.

[2] The amount consumed limited by unpleasant taste, odor and/or color.

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT

October 2, 2006

Panel Members

Robert Nicolosi, Ph.D.

I. Glenn Sipes, Ph.D.

John Thomas, Ph.D., F.A.T.S.

DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT

TABLE OF CONTENTS

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT

1. EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel),[1] qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by The Nisshin OilliO Group, Ltd. (hereafter referred to as Nisshin) to determine the Generally Recognized As Safe (GRAS) status, based on scientific procedures, of MLCT-Oil. MLCT-Oil is to be used as an ingredient to provide consumers with a supplementary source of oil in their diets, at an estimated total daily consumption of MLCT-Oil at the mean and 90th percentile of approximately 11 and 31 g/day, respectively. In particular, the Expert Panel has evaluated the proposed use of MLCT-Oil at specified levels in the replacement of common vegetable oil used in cooking, salad dressings, margarine spreads, and frozen dinners, as described in this document. A comprehensive search of the scientific literature was conducted for safety and toxicity information on medium and long chain triacylglycerols and related compounds and, along with supporting documentation, was made available to the Expert Panel. In addition, the Expert Panel independently evaluated materials submitted by Nisshin, and other materials deemed appropriate and necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed to the decision described herein.

2. INTRODUCTION

Examination of the trends in the prevalence of overweight individuals and increased body mass index of the US adult population, documents a substantial increase in obesity among US adults during the period of 1988 to 1991, with a mean body mass index increase from 25.3 to 26.3, and a mean body weight increase of 3.6 kg, for adults 20 to 74 years of age (Kuczmarski *et al.*, 1994). In advanced countries, the increase in obesity (defined as those people with a BMI[2] of $\geq$30 kg/m^2, by the WHO classification) has become a critical issue in human health (Allison *et*

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[1] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.

[2] BMI = Body Mass Index

al., 1999) particularly in the US, where one person in every three is already obese and still continues to add weight. Obesity not only limits the activities of daily living, but it is frequently associated with life-style related diseases such as hypertension, hyperlipemia and diabetes mellitus, as well as a risk factor for atherosclerosis, as described in the National Cholesterol Education Program (NCEP) – Adult Treatment Panel III (NCEP, 2002). Reports indicate that annually, up to 280,000 Americans die from obesity-related diseases (Allison *et al.*, 1999; Flegal *et al.*, 2005).

There are various causes of obesity, although the basic underlying principle is that the daily intake of calories exceeds the body's need. The amount of dietary fat is a matter of particular importance, as typical fat metabolism provides 9 kilocalories of energy, while carbohydrates and protein only contain 4 kilocalories of energy. Despite various administrative measures, such as the Nutrition Labeling and Education Act (NLEA)[3] and the Dietary Supplement Health and Education Act (DSHEA),[4] to help heighten America's awareness of maintaining good health, the *percentage* of obese Americans continues to grow. In the modern diet, fats and oils are indispensable food ingredients for uses other than basic nutritive value, such as, but not limited to, the addition of fat or oil to improve the flavor and aroma of food, and to intensify the taste of added spices and extracts, thus making it difficult to reduce fat intake.

Triacylglycerols that are modified or restructured from natural oils and fats, having special functionality and/or nutritional properties for edible or pharmaceutical (*e.g.*, excipient) purposes are commonly referred to as structured triacylglycerols (Akoh, 1995; Hoy and Xu, 2001). In this document, structured triacylglycerols are defined as those oils modified to contain fatty acids of different chain lengths incorporated into a specific position on the glycerol backbone.

MLCT-Oil is an edible cooking oil composed of a glycerol backbone with medium chain[5] and long chain[6] fatty acids randomly bound to the sn1, sn2 or sn3 positions (Figure 1).[7] Because

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[3] http://www.fda.gov/ora/inspect_ref/igs/nleatxt.html; site visited August 8, 2006.

[4] http://www.cfsan.fda.gov/~dms/dietsupp.html; site visited August 8, 2006.

[5] *Medium chain fatty acids* used for MLCT-Oil production are defined in this GRAS dossier those fatty acids that contain between 8 (C8) and 10 (C10) carbons (*i.e.*, capric or caprylic acids).

[6] Long chain fatty acids are those that contain 16 carbons or more (*i.e.*, palmitic, linoleic, or arachidonic acids).

[7] Throughout the rest of this document, the proposed ingredient will be referred to as MLCT-Oil.

medium chain fatty acids (MCFA) and long chain fatty acids (LCFA) are randomly attached, MLCT-Oil medium (M) and long (L) chain triacylglycerol (MLCT) can have up to six possible configurations, *i.e.*, (1) L-L-L, (2) L-L-M, (3) L-M-L, (4) L-M-M, (5) M-L-M, and (6) M-M-M. Nisshin has performed fatty acid and acylglycerol compositional analysis on the MLCT-Oil that further defines the identity of the finished product. This dossier is a summary of the scientific evidence that supports the general recognition that MLCT-Oil is safe for human consumption as a food ingredient.

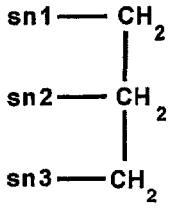

Figure 1. Chemical structure of a triacylglycerol backbone. Sn1, sn2 and sn3 refer to the positions for the three fatty acid molecules to attach to the triacylglycerol backbone (Babayan, 1987).

MCFAs utilized to produce MLCT-Oil are derived directly from medium chain triacylglycerols (MCTs), which are produced from high MCFA-containing oils, such as coconut and palm kernel oils, and contain about 13% and 8% C8 and C10 fatty acids, respectively. MCTs first became commercially available in 1955, and were utilized in the treatment of patients with various fat malabsorption syndromes, surgical patients, cancer patients, and preterm infants (Babayan, 1987). A GRAS affirmation petition for MCTs was accepted for filing on June 17, 1994, with the common name of Captrin (Heydinger and Nakhasi, 1996). Furman *et al.* (1965) noted that MCTs in a normal diet had little effect on cholesterol levels, while a saturated fat (from butter) raised cholesterol levels and unsaturated fats, such as corn oil, decreased cholesterol levels. Long chain triacylglycerols (LCT) are known to contain 9 kcal/g of energy, while the gross energy content for MCTs has been determined through bomb calorimetric methods at 8.3 kcal/g. However, a review of the calculated energy loss during utilization of MCTs concluded that the net usable energy value for metabolism of MCTs was actually closer to 6.8 kcal/g, with increased energy expenditure occurring after the consumption of MCT (Swanson, 1996; Ingle *et al.*, 1999; St-Onge and Jones, 2002).

2.1. Fatty Acid Composition

Table 1 presents the fatty acid composition analysis of MLCT-Oil. MLCT-Oil contains (as a *percent* of the total fatty acid content) predominantly monounsaturated fatty acids (up to 55.8%), and nearly equal portions of saturated (up to 19.7%), and polyunsaturated (up to 23%) fatty acids (Nisshin, 2006c). By far, the predominant fatty acid in MLCT-Oil is oleic acid (up to 54.2%). Linoleic acid is present at an appreciable quantity (*i.e.*, up to 17.0%), while the remaining fatty acids are present at less than 10% of the total fatty acids. MLCT-Oil is comprised of approximately 12% medium chain fatty acids and 85.2% long chain fatty acids (based on the total amount of fatty acids present in MLCT-Oil).

Table 1. Fatty acid composition of MLCT-Oil (Nisshin, 2006c)

Fatty Acid	M/L	*Percent* (%)*	Fatty Acid	M/L	*Percent* (%)
Caprylic acid (C8:0)	M	8.5-9.1	Linoleic acid (C18:2)	L	16.1-18.8
Capric acid (C10:0)	M	2.7-2.8	Linolenic acid (C18:3)	L	5.4-10.3
Lauric acid (C12:0)	L	ND	Arachidic acid (C20:0)	L	0.4-0.6
Myristic acid (C14:0)	L	ND	Gadoleic acid (C20:1)	L	0.9-1.2
Palmitic acid (C16:0)	L	3.2-4.0	Behenic acid (C22:0)	L	0.2-0.4
Palmitoleic acid (C16:1)	L	0.1-0.2	Erucic acid (C22:1)	L	0.1-0.3
Stearic acid (C18:0)	L	1.6-1.8	Lignoceric acid (C24:0)	L	0.1-0.2
Oleic acid (C18:1)	L	49.0-54.2	Nervonic acid (C24:1)	L	0.1-0.3

*As a *percent* of the total fatty acid content; M= medium chain fatty acid; L= long chain fatty acid; ND= not detected; MLCT= medium- and long-chain triacylglycerol

2.2. Acylglycerol Composition

Many of the commercially important fats and oils of animal and plant origin are composed of triacylglycerols, consisting of a trihydric alcohol glycerol typically esterified with long-chain fatty acids (Christie, 2005). Because MLCT-Oil is a manufactured vegetable oil (*i.e.*, not extracted from a plant part (*e.g.*, leaf) or plant product (*e.g.*, bean)), it is possible that the final product not only contains triacylglycerol, but also di- and mono- acylglycerol molecules. Compositional analysis indicates that MLCT-Oil contains 95.88%-97.32% triacylglycerol molecules, while the remainder of the glycerol molecules are diacylglycerols (2.68%-4.12%) (Nisshin, 2006e).

Of the six possible triacylglycerol configurations, MLCT-Oil is composed of those configurations that contain at least two long chain fatty acids bound to the glycerol backbone

(*i.e.*, up to 92.4%) (Table 2). Less than 10% of MLCT-Oil is of triacylglycerol molecules that contain less than two long chain fatty acids.

Table 2. Triacylglycerol composition of MLCT-Oil (Nisshin, 2006g)

Fatty Acid	*Percent* (%)
L-L-L	49.5-52.7
L-L-M or L-M-L	37.3-39.6
L-M-M or M-L-M	8.6-9.3
M-M-M	0.1-0.2

M=medium chain fatty acid; L=long chain fatty acid; MLCT=medium- and long-chain triacylglycerol

2.3. Regulatory Status

MLCT-Oil (containing approximately 12% MCFA) manufactured *via* transesterification from LCT (from vegetable oils such as rapeseed oil) and MCT (from coconut and/or palm kernel oil) to produce a novel food product, was granted "Food for Specified Health Use" (FOSHU) status by the Ministry of Health, Labor, and Welfare in Japan on December 6, 2002. FOSHU is defined as a food that has beneficial, effective ingredients added to help in the maintenance of a healthy body condition. The standards for such foods include criteria stating that the food should be expected to improve one's diet, as well as the maintenance and or enhancement of health. In addition, the food (or its constituents) should be safe to eat, with no significant loss in its nutritive constituents, in comparison with those normally contained in similar foods or food ingredients. MLCT-Oil is sold in Japan as a cooking oil, with the total sales since it was launched in 2003 at approximately $300 million dollars.[8]

3. DESCRIPTION, MANUFACTURING PROCESS AND SPECIFICATIONS

3.1. Description

The substance of this GRAS monograph is MLCT-Oil, which consists of medium and long-chain triacylglycerols. MLCT-Oil is an edible vegetable oil manufactured from common edible vegetable oils containing LCFA, such as those found in rapeseed, soybean, cottonseed, sunflower seed, peanut, olive, corn, safflower, rice bran, and sesame seed oils, while MCT is

[8] Nisshin OilliO Group, LTD. (Personal communication), August 30, 2006.

produced from coconut and palm kernel oils. Specific ratios of edible vegetable oil and MCT produced from edible oils are combined with a lipase utilized to promote a randomized ester exchange (Hoy and Xu, 2001) such that the MLCT-Oil produced is composed of both medium- and long-chain triacylglycerols (MLCT). The MCFA of MLCT-Oil consists only of caprylic and capric fatty acids. Since MLCT-Oil has been defined as including at least one LCFA molecule in a triacylglycerol, only a maximum of two MCFA will be included in the triacylglycerol molecule. The physical and chemical properties of MLCT-Oil are given in Table 3.

3.2. Manufacturing Process and Specifications of MLCT-Oil

3.2.1. Manufacture of MLCT-Oil

MLCT-Oil is manufactured in accordance with the manufacturing control standards and the quality control standards of the International Organization for Standardization (ISO) at the manufacturing facility of Nisshin Oillio Group, LTD. MLCT-Oil is manufactured from common edible vegetable oils (*e.g.*, rapeseed, soybean and cottonseed) and medium chain triacylglycerol (MCT) derived from edible coconut or palm kernel oil, by random transesterification using a bacterium-derived lipase (APPENDIX A). All of the constituents in MLCT-Oil are either approved food ingredients (GRAS), or are normal constituents found in commonly consumed foods at similar concentrations. The lipase has been deemed GRAS, and is derived from a source organism considered safe, as recommended by Pariza and Johnson (Pariza and Johnson, 2001) (APPENDIX A).

The two starting materials (*i.e.*, common vegetable oil and MCT) are produced by traditional manufacturing methods. For common vegetable oil (*e.g.,* rapeseed oil), manufacturing includes extraction, steam distillation, degumming, de-acidification (*i.e.*, alkali), bleaching, and deodorizing.[9] MCT is produced from coconut or palm kernel oil by saponification or hydrolysis[10] that produces mixed fatty acids. The fatty acid mixture is subjected to fractional distillation to isolate MCFA (*i.e.*, caprylic and capric acids). The MCFA are esterified with glycerin to produce a crude MCT product, which is purified using traditional oil processing procedures.

[9] Erickson, D.R. (1995) *Practical Handbook of Soybean Processing and Utilization.* AOCS Press and the United States Soybean Board, Champaign, IL and St. Louis, MO.

[10] High pressure steam

Enzymatic esterification is initiated by mixing vegetable oil with MCT at the appropriate temperature to produce a MLCT crude oil product (Figure 2). Esterification is initiated by allowing the vegetable oils (containing either LCFA or MCFA as raw materials) to be exposed to the lipase. The crude oil product is filtered to remove lipase (*i.e.*, lipase free MLCT). Then, the product is subjected to traditional oil processing (*i.e.*, de-acidification, bleaching, deodorizing, mixing, packing and analysis) to produce the final MLCT-Oil product. The MLCT-Oil product is washed with hot water during the de-acidification process, ensuring the complete removal of lipase from the product. To confirm that the lipase has been removed, acid production is measured, as residual lipase would contain hydrolytic activity and produce free fatty acids. Therefore, acid production is measured after agitation of a mixture of MLCT-Oil and water at the appropriate temperature, which facilitates any fatty acid production from residual lipase. The acid value after agitation is compared with the value prior to agitation. Significant increases in the acid value would warrant re-washing with hot water to remove any remaining lipase. The content of MCFA in MLCT-Oil is determined by the blending quantity of raw material used in the manufacturing process (Nisshin, 2006d). The manufacturer's quality assurance department will analyze the MLCT-Oil, and if the product does not meet specifications, the product will be reformulated to meet specifications presented in Table 3, or the MLCT-Oil batch will be discarded.

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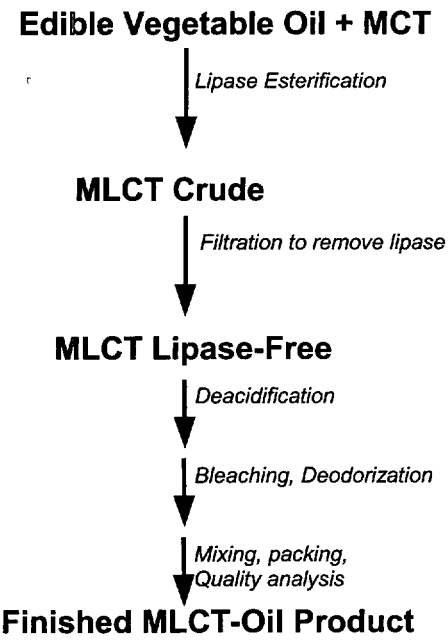

Figure 2. Detailed flow chart for the manufacture of MLCT-Oil (Nisshin, 2006d)

3.3. Summary of MLCT-Oil Identity and Manufacturing

The composition of MLCT-Oil is well characterized, thus its identity is well understood. It is composed of a glycerol with randomly bound medium and long chain fatty acids. The fatty acids are derived from common edible oils rich in free medium and long chain fatty acids. Compositional analysis indicates that the fatty acids present in MLCT-Oil are the type commonly found in other edible oils (Babayan, 1987; Kasai *et al.*, 2003)

The manufacturing process is important because not only must the final product be of suitable purity for consumption, but the materials used to produce MLCT-Oil must also be food grade. Commonly consumed vegetable oils (*e.g.*, rapeseed, soybean, cottonseed, and coconut or palm kernel oils) are utilized in the manufacture of MLCT-Oil. MLCT-Oil is manufactured by an esterification method. The oil contains approximately 12% MCFA, with the balance made up of LCFA (Table 2). MLCT-Oil specifications are provided in Table 3 (Nisshin, 2006a). As part of the specifications to identify MLCT-Oil, the fatty acid composition is presented in Table 1.

Table 3. Specifications of MLCT-Oil (Nisshin, 2006a)

Analysis	Method	Specification	Batch Analysis Results (n = 5) Range	Average
Acid value	JOCS Official Methods 2.3.1. - 1996	Less than 0.15%	0.03 – 0.04%	0.04%
Appearance		Clear (Liquid oil equivalent in appearance to edible vegetable oils)	NA	NA
Arsenic	Flameless atomic absorption[a]	Less than or equal to 0.1 ppm	Passes	Passes
Color	JOCS Official Methods 2.2.1.1. - 1996	10Y/1.0R and below (133.4 mm cell)	2.6 – 6.0Y/0.2 – 0.6R	Passes
Heavy metals	Colorimetric method[a]	Less than or equal to 0.1 ppm	ND	ND
Iodine value	JOCS Official Method 2.3.4.1. - 1996	85-118	102.2 – 103.8	103.3
Peroxide value	JOCS Official Method 2.5.2.1 - 2003	Less than 10 meq/kg	0.0-0.6 meq/kg	0.3 meq/kg
Refractive Index	JOCS Official Method 2.2.3. - 1996	1.4665-1.4715	1.4686 – 1.4688	1.4687
Unsaponification value	JOCS Official Method 2.4.8. - 1996	Not more than 1.5%	0.84 – 1.02	0.91
Soap	AOCS Official Method Cc17-95	Less than or equal to 5.0 ppm	0.0	0.0
Moisture	JOCS Official Method 2.1.3.4. - 1996	Less than or equal to 0.2%	0 – 0.2%	0.01%
Medium chain fatty acid content (%)	D-46[a]	11.1 – 12.5 g/100 g	11.8 – 12.3%	12.1%

[a]Nisshin Method, SOP available on request; AOCS = American Oil Chemists' Society; JOCS = Japan Oil Chemists' Society; MLCT=medium- and long-chain triacylglycerol; NA = Not appropriate; ND = Not detected; ppm= parts *per* million; R = Red; Y = Yellow

3.4. Stability

MLCT-Oil has been tested for stability at room temperature. One lot comprised of six batches of MLCT-Oil was placed in various plastic or metal containers commonly used for common edible vegetable oil and sealed, then stored at room temperature, either in the light or the dark for twelve months and analyzed for degradation. Table 4 and Table 5 represent typical results of sealed storage in the light or dark for twelve months in ethylene-vinylalcohol copolymer/polyethylene bags, aluminum bottles, tin cans, polyethylene terephthalate (PET) bottles, nylon/polyethylene (PE) pouches, or steel cans (Nisshin, 2006f). Under these conditions, the acid values, peroxide values, water content, and color remained similar to those of the control edible vegetable oil (prepared by mixing 70% rapeseed oil and 30% soybean oil).

Table 4. Stability of MLCT-Oil in the light at room temperature (Nisshin, 2006f)

Item	Test or control	Initial	Month 12	Reference
Acid values	MLCT-Oil	0.03%	0.06%	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.05%	0.09%	2.3.1. - 1996
Peroxide values	MLCT-Oil	0.05 ppm	3.25 ppm	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.25 ppm	3.70 ppm	2.5.2.1 - 2003
Moisture	MLCT-Oil	0.02%	0.06%	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.02%	0.04%	2.1.3.4. - 1996
Color	MLCT-Oil	4.3Y/0.5R	3.0Y/0.3R	JOCS Official Method
	70% Rapeseed/30% Soybean oil	6.4Y/0.8R	3.9Y/0.5R	2.2.1.1. - 1996
Medium-chain fatty acid	MLCT-Oil	12.0%	11.7%	D-46[a]

[a]Nisshin Method, SOP available on request; JOCS = Japan Oil Chemists' Society; MLCT=medium- and long-chain triacylglycerol

Table 5. Stability of MLCT-Oil in the dark at room temperature (Nisshin, 2006f)

Item	Test or control	Initial	Month 12	Reference
Acid values	MLCT-Oil	0.03%	0.05%	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.05%	0.08%	2.3.1. - 1996
Peroxide values	MLCT-Oil	0.05 ppm	2.77 ppm	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.25 ppm	3.10 ppm	2.5.2.1 - 2003
Moisture (%)	MLCT-Oil	0.02%	0.06%	JOCS Official Method
	70% Rapeseed/30% Soybean oil	0.02%	0.05%	2.1.3.4. - 1996
Color	MLCT-Oil	4.3Y/0.5R	6.8Y/0.7R	JOCS Official Method
	70% Rapeseed/30% Soybean oil	6.4Y/0.8R	9.9Y/1.1R	2.2.1.1. - 1996
Medium-chain fatty acid (%)	MLCT-Oil	12.0%	11.6%	D-46[a]

[a]Nisshin Method, SOP available on request; JOCS = Japan Oil Chemists' Society; MLCT=medium- and long-chain triacylglycerol

To evaluate the oxidation and the stability of MLCT-Oil when heated, heat stability tests (200°C, with exposure to the atmosphere for 30 minutes) and oxidation stability tests were performed. Table 6 shows that MLCT-Oil was equal or superior to the mixture of rapeseed and soybean oils (70%:30%, respectively) used as a control, with respect to the acid, peroxide, and color values. The baseline *percentages* of MCFA contained in the oil after heating were nearly equal to the values before heating. This data indicates that MLCT-Oil is stable in storage for twelve months, and is stable during heating to 200°C for 30 minutes (Nisshin, 2006f).

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Table 6. Stability of MLCT-Oil when heated (Nisshin, 2006f)

Item	Test or control	Before heating	After heating	Reference
Acid values	MLCT-Oil	0.03%	0.15%	JOCS Official
	70% Rapeseed/30% Soybean oil	0.05%	0.15%	Method 2.3.1-1996
Peroxide values	MLCT-Oil	0.05 ppm	1.95 ppm	JOCS Official
	70% Rapeseed/30% Soybean oil	0.25 ppm	2.38 ppm	Method 2.5.2.1-2003
Color	MLCT-Oil	4.3Y/0.5R	6.8Y/0.7R	JOCS Official
	70% Rapeseed/30% Soybean oil	6.4Y/0.8R	9.9Y/1.1R	Method 2.2.1.1-1996
Medium-chain fatty acid	MLCT-Oil	12.0%	12.2%	D-46[a]

[a]Nisshin Method, SOP available on request; JOCS = Japan Oil Chemists' Society; MLCT=medium- and long-chain triacylglycerol

4. ESTIMATED DAILY INTAKE

Key to any food safety risk assessment process is an understanding of the anticipated consumption of the food ingredient(s) in question as an index of consumer exposure at the proposed use level(s) for the ingredient(s). MLCT-Oil is an edible vegetable oil that is equivalent in quality, physical properties, flavor, and taste, to common vegetable oils. As such, MLCT-Oil is intended for use as a substitute of edible vegetable oils consumed in the US population, and not to increase the overall consumption of edible vegetable oils. MLCT-Oil will be added to the foods presented in APPENDIX B, and the weighted mean and 90th percentile consumption of MLCT-Oil is calculated at 11.4 and 30.9 g/day, respectively (approximately equivalent to 183.4 and 459.9 mg/kg/day, respectively, based on the weights of the sample population queried for this consumption analysis). The fatty acid composition of edible cooking oils consumed in the US population is equivalent to that found in MLCT-Oil; therefore, the dietary fatty acid consumption does not change with ingestion of MLCT-Oil.

4.1. Self-limitation of Use of MLCT-Oil

MLCT-Oil is equal to common edible vegetable oils in quality, properties, and flavor. MLCT-Oil can be used in place of the edible vegetable oil conventionally used in processed food and as home cooking oil. The quantity of MLCT-Oil use is not considered self-limiting (*i.e.*, the amount consumed limited by unpleasant taste, odor, and/or color) any more than conventional edible vegetable oil.

5. ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION (ADME)

5.1. Digestion, Absorption, and Distribution

MLCT-Oil is a food ingredient source of medium- and long-chain triacylglycerols, which may be derived from coconut or palm kernel oil, and rapeseed oil, respectively. Significant

differences in the metabolism of long-chain fatty acids (LCFA) derived from LCT (*e.g.*, soybean, cottonseed, and rapeseed oils), and medium-chain fatty acids (MCFA) derived from MCT, must be considered when analyzing the metabolism of MLCT-Oil. To this end, the digestion, absorption, and metabolism of LCT and MCT will be reviewed below.

Following ingestion, LCT are first acted upon by buccal, gastric, pancreatic, and intestinal lipases that catalyze the breakdown of the triacylglycerol to form two free long-chain fatty acids (LCFA) and an sn2 monoacylglycerol (*i.e.*, the middle fatty acid remains attached to the glycerol backbone) (Hoy and Xu, 2001). LCT consumption regulates pancreatic triacylglycerol lipase mRNA expression and lipase activity, with the pancreatic triacylglycerol lipase protein expression restricted to enterocytes throughout the small intestine in the rat (Mahan *et al.*, 2001). For transport through the blood, the LCFA and sn2 monoacylglycerols must be packaged into micelles. Micelles contain important bile salts, phospholipids and other emulsifiers, necessary for their formation and binding to enterocytes. After micelle incorporation, absorption into the intestinal mucosa can occur throughout the small intestine. The sn2 monoacylglycerol is preferentially absorbed over the free fatty acid. In the mucosa, the sn2 monoacylglycerol serves as a template for triacylglyceride formation, and the LCFA are converted into acyl-CoAs in the presence of acyl-CoA synthetase (an enzyme specific for fatty acids with more than 12 carbon atoms). Once formed, the LCFA acyl-CoA's are packaged into micelles, containing emulsifiers, bile salts, and phospholipids, followed by reesterification back onto the sn2 monoacylglycerol to reform triacylglycerols. Triacylglycerols are then packaged by the intestinal cells into lipoprotein complexes called chylomicrons, which are secreted into the lymphatic system and eventually enter the systemic circulation (Figure 3). Carnitine is required for transport of the LCFA across the mitochondrial membrane to be oxidized for energy at the target tissue, or for storage as a triacylglycerol (Bach and Babayan, 1982; Bell *et al.*, 1997). Chylomicrons containing long-chain triacylglycerols enter the circulation and are hydrolyzed by lipoprotein lipase (from capillary surfaces) to release fatty acids, which are then taken up by adipose tissue and reesterified into triacylglycerols for storage and later release as an energy source (IoM, 2002; Mu and Porsgaard, 2005).

MCT are processed differently from dietary fats that contain only LCT, as described in the previous paragraph. MCT are also acted upon by buccal, gastric, intestinal, and pancreatic

lipases, to form free fatty acids and glycerol in the small intestine. As medium-chain fatty acids (MCFA) are more hydrophilic than LCFA, the majority of MCFA do not require micelle-containing bile salts or chylomicron formation, but are directly absorbed into the liver *via* the portal vein, rather than through the thoracic duct lymphatic system (Figure 3). However, when humans consume a diet rich in MCT (approximately 40% of the diet composed of MCT), Swift *et al.* (1990) reported that MCFA comprised 8% of the total chylomicron triglyceride fatty acids, indicating that excess MCFA may be incorporated into chylomicrons and enter the lymphatic system. Reesterification of MCFA to MCT does not occur in the intestinal mucosal cell, as in LCT absorption. Once in the hepatocytes, transport of the MCFA across the mitochondrial membrane occurs *via* a carnitine-independent mechanism (although the carnitine-dependent transfer is a rate-limiting step for LCFA); with the MCFA predominantly oxidized to CO_2, acetate and ketones (Schwab *et al.*, 1964; Wiley and Leveille, 1973; Birkhahn and Border, 1981; Babayan, 1987; Bell *et al.*, 1997).

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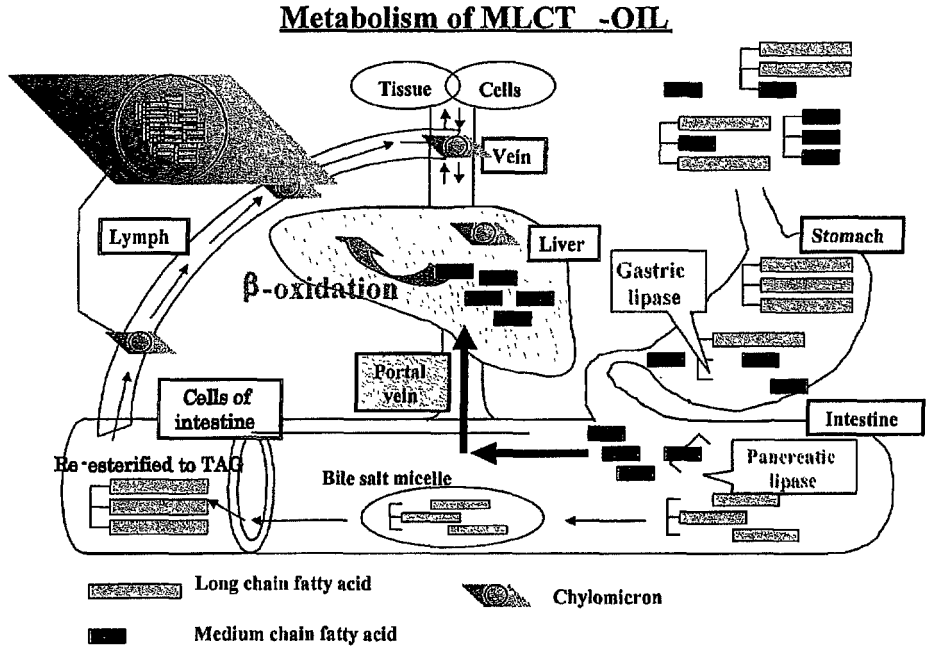

Figure 3. Metabolism of medium-chain and long-chain triglycerides

The absorption of dietary LCFA has been investigated during the administration of a structured MLCT to Sprague Dawley rats when the positional distribution of the MCFA was altered (Carvajal *et al.*, 2000; Mu and Hoy, 2000). Two types of structure-specific fats were prepared by enzymatic transesterification with a 1,3-specific lipase. One contained MCFA in the sn1-3 and linoleic acid in the sn2 positions [sn1-3 MCFA-structured] (providing approximately 40% of fatty acids as MCFA), and another contained MCFA in the sn2 and linoleic acid in the sn1-3 [sn-2MCFA-structured] positions (providing approximately 20% of fatty acids as MCFA). Equal amounts (10.3 g *per* 100 g of diet) of the synthetic fats and cocoa butter were fed to rats for four weeks to provide 40% of the energy requirement as fat. Long-chain saturated fatty acids were the predominant fatty acids excreted into the feces. No significant differences were observed for the maximal intestinal absorption of linoleic acid when the chain length of the MCFA varied. The rats fed the diet containing sn1-3 MCFA-structured fat had a lower fecal weight than those fed the corresponding sn-2 MCFA-structured fats ($P<0.05$). The proportion of MCFA in the serum, liver, and adipose tissue triacylglycerols was not affected by the MCFA distribution of the dietary fats. However, the accumulated lymphatic transport of MCFA

increased with increasing carbon chain length. The recoveries of caprylic acid (8:0), capric acid (10:0), and lauric acid (12:0) from lymph samples were 7.3 ±0.9, 26.3 ±2.4, and 81.7 ±6.9%, respectively, with no significant differences observed for the maximal intestinal absorption of linoleic acid. This information indicates that the chain length of the MCFA in the structured fat does not affect the maximal intestinal absorption of LCFA, while the distribution of FA between the lymphatics and the portal vein does reflect FA chain length (Mu and Hoy, 2000).

Additional research by Carvajal *et al.* (2000) noted that the positional distribution of MCFA in the dietary fat had no significant effect on the lymph flow, triacylglycerol output, phospholipid output, lipid composition of chylomicrons, or the particle size. In this study, male Sprague Dawley rats were fed a diet that provided 40% of the energy as fat from structured MLCT containing approximately 30% MCFA for three weeks, then analyzed for chylomicron formation and lymphatic transport. Lymph chylomicrons contained significant levels of MCFA, with the positions of MCFA in the structured MLCT having no significant effect on the chemical composition and the sizes of chylomicrons. These data in rats indicate that some MCFA in the dietary triacylglycerol in the rat is transported into the lymphatic system, with the positional distribution of the MCFA well preserved in the chylomicron triacylglycerol (Carvajal *et al.*, 2000).

Straarup and Hoy (2000) found that administration (*via* gavage) of an MLCT containing capric acid (10:0) in the sn1 and 3 positions, influenced the absorption and lymphatic transport of fatty acids in male Wistar rats. The lymphatic transport of fatty acids during 24 hr after administration of different oils was examined in the mesenteric lymph duct, and it was found that the capric fatty acid was rapidly absorbed, with maximum transport 2 hr after administration of this MLCT. Maximum absorption of oleic acid [18:1 (n-9)] and linoleic acid [18:2 (n-6)] occurred after 3 hr for MLCT, and after 5-6 hr for the rapeseed oil control. However, the accumulated lymphatic transport (in mg) of total fatty acids (after a 24 hr period) did not differ between groups. Interestingly, the recoveries of 18:1(n-9), 18:2(n-6), and linolenic acid [18:3(n-3)] in rats fed MLCT were higher than those rats fed rapeseed oil ($P<0.05$). This information indicates that MLCT is readily hydrolyzed and absorbed, and may influence the absorption of long-chain fatty acids into the lymphatic system.

Vistisen *et al.* (2003) calculated the lymphatic recovery of dietary MLCT and separated the exogenous and endogenous lymphatic fatty acids, by comparing the lymphatic transport of fatty acids after administration of radiolabeled ML*M (where L = linoleic acid [18:2(n-6)], M=8:0, and * = ^{13}C-labeled fatty acids), ML*L*, L*L*L*, and M*M*M*. The recoveries of ^{13}C-labeled lymphatic linoleic acid [18:2(n-6)] tended to be higher 5-8 hr after ML*M and ML*L* administration, compared with L*L*L* administration, but was not statistically significant. However, after 24 hr, similar total recoveries of ^{13}C-labeled 18:2(n-6) were observed between MLCT and LCT dose groups. Administration of MLM significantly increased recovery of ^{13}C-labeled 18:2(n-6) in lymph phospholipids, compared with LLL administration. In addition, recovery of ^{13}C-labeled 18:2(n-6) in lymph free fatty acids 6-7 hr after ML*L* administration was significantly higher than after L*L*L administration. This study indicates that a higher lymphatic recovery of exogenous 18:2(n-6) (linoleic acid) may occur during the first 8 hr following ML*M administration, compared with L*L*L* administration. However, similar overall recoveries of 18:2(n-6) fatty acids occurred when 18:2(n-6) fatty acids were measured at the 24 hr time point. This study indicates that lipid structures affect the stimulation of endogenous linoleic fatty acid release into the lymphatic system (Vistisen *et al.*, 2003).

Caprenin is a triacylglycerol that is similar to MLCT-Oil, in that it primarily contains medium-chain fatty acids (caprylic (C8:0) and capric (C10:0) acids) and a long-chain fatty acid (behenic (C22:0) acid). Caprenin has been evaluated for digestion and absorption in rats (Webb and Sanders, 1991). After male and female Sprague Dawley rats (n = 40) were surgically implanted with thoracic duct catheters, each animal was administered (*via* gavage) 5 ml of a modified emulsion that contained 31.5% (w/w)[11] water, 18.5% (w/w) skim milk solids, 20.0% sucrose, and 30.0% fat and/or water. The composition of the emulsions resulted in each animal receiving either a fat-free control, or 1.5 g of caprenin, coconut oil, or peanut oil (coconut and peanut oils are a source of MCFA and LCFA, respectively). The fatty acid compositions of these triacylglycerols are provided in Table 7. Lymph was collected 24 hr after dosing, and frozen at –20°C until analyzed for lymph triglyceride and fatty acid content. Aliquots of the administered

[11] w/w = Weight/weight

oils were also analyzed for *in vitro* hydrolysis by a modified porcine pancreatic lipase and *in vitro* digestibility.

Table 7. Fatty acid composition of test oils (Webb and Sanders, 1991)

Fatty acid composition		Fatty acid concentration (weight %)		
		Caprenin	Coconut oil	Peanut oil
8:0	Caprylic	23.3	7.1	ND
10:0	Capric	24.9	5.8	ND
12:0	Lauric	0.5	46.3	ND
14:0	Myristic	ND	19.2	ND
16:0	Palmitic	ND	9.8	10.2
18:0	Palmitoleic	0.4	2.9	2.3
18:1	Stearic	ND	7.0	47.3
18:2	Oleic	ND	1.9	31.8
20:0	Arachidic	3.6	ND	1.3
20:1	Gadoleic	ND	ND	1.5
22:0	Behenic	46.6	ND	3.6
24:0	Lignoceric	0.7	ND	2.0
Total		100.0	100.0	100.0

ND = Not detected

In vitro digestion results showed that 75-92% of either caprenin, peanut oil, or coconut oil were hydrolyzed during 30 min of enzymatic lipolysis. All administered triglycerides were absorbed by the lymphatic system by both male and female rats (Table 8). Therefore, as absorption occurred, *in vivo* digestion can be inferred, since it is generally recognized that long-chain triacylglycerol molecules must be enzymatically hydrolyzed before lymphatic uptake can occur (Greenberger *et al.*, 1966).

Table 8. Lymphatic recovery of administered oils in male and female rats after correction for endogenous lipids (Webb and Sanders, 1991)

Treatment Group	Lymph lipids (24 hr)[a] (mg)	Net increase over control (mg)	Recovery relative to a 1.5 g dose (%)
Control (fat-free)	284.3±22.1	NA	NA
Caprenin	438.2±23.3[b]	153.9	10.3
Coconut oil	1051.5±58.8[c]	767.2	51.1[f]
Peanut oil	1393.7±64.6[d]	1109.4	74.0

[a]Values are means ± SEM of 5 male and 5 female rats; [b]$P<0.05$ from the Control oil; [c]$P<0.05$ from Control and Caprenin oils; [d]$P<0.05$ from Control, Caprenin, and Coconut oils; [f]$P<0.05$ from Caprenin and Coconut oils; NA=Not applicable

Caprylic, capric, and behenic acids were absorbed from all three administered oils (when present), and portions of each dose were recovered in the extracted lymphatic triglycerides (Table 9). This study demonstrates that caprenin is enzymatically hydrolyzed by pancreatic

lipase to typical end products, like those found in coconut and peanut oils, and are absorbed either lymphatically or portally, based on fatty acid chain length. These lymphatically absorbed digestion products are resynthesized into triglycerides and secreted into the thoracic lymph duct. Extensive hydrolysis of medium chain triglycerides occurs in the intestinal lumen before absorption into the portal vein, in the form of MCFA (Hashim *et al.*, 1964).

Table 9. Lymphatic recovery of C8:0, C10:0, and C22:0 fatty acids in male and female rats (Webb and Sanders, 1991)

Treatment Group	Lymph C8:0 (mg)[b]	Recovery[a] (%)	Lymph C10:0 (mg)	Recovery (%)	Lymph C22:0 (mg)	Recovery (%)
Caprenin	13.5±0.9	3.9	66.6±4.5	7.8	78.4±6.3	11.2
Coconut oil	2.1±0.3	2.0	14.2±1.1	16.3	ND	ND
Peanut oil	ND	ND	ND	ND	29.9±1.8[c]	55.4[c]

[a]24 hr recovery relative to the administered dose; [b]Values are means ± SEM of 5 male and 5 female rats; [c]$P<0.05$ from Caprenin value; ND=Not detected

5.2. Metabolism

Medium-chain fatty acids are preferentially oxidized in the liver (*via* a carnitine-independent transport system), which results in the formation of large amounts of acetyl CoA (Figure 3). Acetyl CoA enters the citric acid cycle and is completely oxidized to carbon dioxide. Excess CoA condenses to form ketone bodies (*e.g.*, acetoacetate and β-hydroxybutyrate) that are released into the circulation and may be used as an alternate energy substrate to glucose for the brain and muscle. The production of ketone bodies through the consumption of MLCT would not be high enough to form toxic levels of ketone bodies (Dias *et al.*, 1990; IoM, 2002). Christensen *et al.* (1989) found that when *in vitro* hepatocyte mitochondrial fatty acid oxidation is down-regulated, as may occur during carbohydrate consumption, MCFA can be partially reformed into LCFA by peroxisomal β-oxidation, followed by C_{16} synthesis, and ultimately stored as triacylglycerols. In contrast, MLCT has also induced hepatic fatty acid oxidation enzyme activities 30 minutes after oral administration to male Wistar rats (7300 mg/kg bw),[12] without increasing lipogenic enzyme activity, indicating that MLCT tends to cause lipolysis by β-oxidation, rather than lipogenesis (Shinohara *et al.*, 2002). Rats fed 20% MCT exhibited lower serum and liver cholesterol levels than control rats or rates fed 20% coconut oil or corn oil (Kritchevsky and Tepper, 1965). Adult rats that received an isoenergetic mixture (*via* gavage) of

[12] bw = Body weight

50% MCT/50% LCT for ten days showed a higher mucosal mass and protein content and increased villus length and crypt depth in the proximal part of the small intestine, compared with rats administered 100% LCT, which are beneficial in states of malabsorption and aging (Galluser *et al.*, 1993).

Previous rat studies with MCT indicated that consumption of MCT at 20% of the diet did not affect survival, fertility, or reproduction, but did decrease the average rat body weights (Kaunitz *et al.*, 1957; Kaunitz *et al.*, 1958). Long-term feeding of MCT to rats decreases plasma lipids and decreases fat deposition in adipose tissue, with a concomitant increase in blood ketone bodies, both early in life and consumption only in adulthood (Wiley and Leveille, 1973; Hashim and Tantibhedyangkul, 1987). Low levels of MCT are found in human milk, and coconut and palm kernel oils (Hilditch, 1956). High MCT diets (approximately 60% of the dietary energy was provided by MCT) fed to rats did not change the plasma insulin or glucagon levels, but reduced energy retention (a calculation derived from the difference between the initial and final body composition), and decreased the daily lipid deposition by 60% (Crozier *et al.*, 1987). This reduced energy retention and lipid deposition has been reported as a result of enhanced thermogenesis induced by MCT, related to the extensive oxidation of MCFA, when compared to LCFA, leading to increased oxygen consumption, and enhanced dissipation of energy as heat (Baba *et al.*, 1982; 1987). Chanez *et al.* (1991) reported that in rats fed a diet containing MCT as 32% of the metabolizable energy, a high lipogenic enzyme activity was observed, similar to rats fed a low-fat, high carbohydrate diet, but significantly higher than rats on a high LCT-containing diet. In addition, the rats on the low-fat and MCT-containing diets had significantly less ($P<0.05$) weight gain over a 21-day period, compared to rats fed the LCT-containing diet (energy values were not significantly different between groups). In an earlier study, rats fed approximately 19.6% MCT and 2.5% safflower oil (for essential fatty acids) for 47 weeks resulted in normal growth and development, although slower weight gains were noted. In addition, rats fed MCT had slightly lower growth rates, caloric efficiency values, less carcass fat, and smaller epidiymal fat pads, than animals fed conventional dietary fats (Harkins and Sarett, 1968). These studies in experimental animal models indicate that MCT consumption does not adversely affect growth and development.

Hwang *et al.* (1993) also found that MCFA were not incorporated into the body fat in the form of MCFA itself. However, the enhanced lipogenic enzyme activities resulted in some fat deposition by the MCT-fed rats, which confirms other work indicating ingestion of MCT stimulates *de novo* synthesis of fatty acids as necessary (Leveille *et al.*, 1967). However, other research has indicated that high levels of MCT consumption (20% w/w in the diet) in Gold Syrian hamsters may be as atherogenic as saturated fat ingestion

(Nicolosi *et al.*, 1998). Administration of 1 or 3 ml MCT (*via* gavage) for 30 days to groups of ten male Wistar rats did not result in adverse effects on weight gain or urinalysis values, although transitory reductions in food intake and other digestive disturbances (*i.e.*, diarrhea) were noted during the first 5-7 days of the trial (Traul *et al.*, 2000).

Foufelle *et al.* (1992) reported that a diet containing long-chain fatty acids inhibited an increase of both the lipogenic enzyme mRNA concentrations and activities that normally occur during the weaning of rats to a high-carbohydrate diet (a high carbohydrate, low fat diet increases lipid formation, as a certain level of lipids is necessary for cellular functions). In contrast, a diet containing MCFA induced a slower, but similar, increase in lipogenic-enzyme mRNA concentrations and activities normally found in rats consuming a high carbohydrate diet, indicating that MCFA affect metabolism in a fashion similar to high carbohydrate, low fat, diets. Animal studies indicate that consumption of MCT results in reduced body weight and smaller fat depots (Lavau *et al.*, 1978; Geliebter *et al.*, 1983). Crozier *et al.* (1987) reported that in rats administered a diet containing 63% of the metabolically energy as either MCT or LCT, the MCT treatment resulted in approximately 13% less energy intake and 30% less weight gain than found in the LCT diet.

In clinical settings, MCT oil was first introduced in the 1950s as a calorie-dense, well-absorbed nutrient to treat patients suffering from impaired absorption of traditional LCT. Extensive research has been conducted on the consumption of MCT, as MCT have been utilized to provide energy to infants and patients with malabsorption syndromes (Holt, 1967; Roy *et al.*, 1975; Chanez *et al.*, 1991). It has also been utilized as a major component of enteral and parenteral diets, in the treatment of cystic fibrosis and fat-induced hyperlipidemia, as part of a ketogenic diet in the treatment of epilepsy, to enhance exercise performance, and to aid in weight

control (Bach and Babayan, 1982; Heydinger and Nakhasi, 1996; Horowitz *et al.*, 2000). Human research indicated that caprylic acid was well absorbed from the gastrointestinal tract and rapidly oxidized (Schwab *et al.*, 1964). Other research indicates that oxidation of MCT is greater than LCT, with oxidation rates for oral [^{13}C]trioctanoate that averaged 34.7%, versus 25.3% for [^{13}C]trioleate (P<0.05) (Metges and Wolfram, 1991). Concurrent ingestion of MCT and LCT slows the metabolism of MCT, but MCT metabolism still remains more rapid than that of LCT, with approximately 90% of MCT converted to carbon dioxide within 24 hours, compared with 45% for LCT (Johnson *et al.*, 1990). In humans, fats containing both medium- and long-chain fatty acids have been reported not to change short-term postprandial appetite sensations or *ad libitum* energy intakes, but do result in higher postprandial energy expenditure and fat oxidation than do conventional fats, thus promoting a negative energy state and fat balance (Bendixen *et al.*, 2002). Conversely, MCT consumption has been reported to increase serum total cholesterol, low density lipoprotein-cholesterol (LDL-C), and triacylglycerol concentrations in male endurance runners (Kern *et al.*, 2000). This discrepancy has yet to be elucidated.

Calabrese *et al.* (1999) administered 71 g of MCT oil to 20 men, and monitored triglyceride values, compared to similar canola oil (a source of LCT) ingestion. Mean triglyceride values after canola oil increased 47 *percent* above baseline (P<0.001), while mean triglyceride values after MCT oil ingestion decreased 15 *percent* from baseline (P<0.001). Significantly more gastrointestinal side effects were noted (*e.g.*, cramping and diarrhea) after MCT oil consumption, compared to canola oil consumption, which the authors contributed to MCT accelerating small-bowel transit time, when compared to control subjects (Calabrese *et al.*, 1999). Overfeeding of MCT increases the thermogenic response to food, compared to overfeeding of LCT (Hill *et al.*, 1989; Dulloo *et al.*, 1996; Noguchi *et al.*, 2002).

Nosaka *et al.* (2003) found that a twelve week ingestion of 5 g MCT in healthy subjects (n = 33) resulted in significant body fat weight, subcutaneous fat, and visceral fat reductions, when compared to a diet containing only LCT. No clinical differences in serum total cholesterol, triglycerides, lipoproteins, plasma glucose, serum insulin total ketone bodies, and aspartate aminotransferase, alanine aminotransferase, and *gamma*-glutamyltranspeptidase activities were noted between groups. Other research indicated that consumption of 40 g MCT/day for four weeks had no adverse effect on serum total cholesterol, very low density lipoprotein cholesterol

(VLDL), low density lipoprotein (LDL) cholesterol, and high density lipoprotein (HDL) cholesterol, and did not cause liver fat accumulation or liver dysfunction in healthy subjects (n = 11) (Nosaka *et al.*, 2002). Ingestion of pure MCT significantly decreases the formation of β-carotene and retinyl palmitate-containing chylomicrons, when compared to LCT ingestion. However, MCT does not affect the rate of intestinal conversion of β-carotene into vitamin A (Borel *et al.*, 1998). This decreased β-carotene-containing chylomicron formation is not expected to occur during the ingestion of MLCT-Oil, as LCT is ingested concurrent with MCT.

5.3. Elimination

Fatty acids are generally oxidized completely to carbon dioxide and water, which are excreted in expired air and urine, respectively (Figure 3). Small amounts of ketone bodies formed during β-oxidation are excreted *via* the urine. Minor amounts of fatty acids are also present in skin and intestinal cells, which are eliminated when the cells die and are sloughed off (IoM, 2002).

6. SAFETY EVALUATION

6.1. Acute Studies

An acute toxicity study was conducted on MLCT-Oil in male and female Wistar rats (Matulka *et al.*, 2006). The study was conducted as a limit test at a dose level of 5000 mg/kg. MLCT-Oil was administered (*via* gavage) to five male and five female rats, and compared to control rats administered an equal amount of control oil (5000 mg/kg bw mixed (7:3) rapeseed and soybean oils). After administration, the general health and survival of the rats were observed for 14 days. Body weights were obtained on the day of test administration and on days 1, 2, 3, 4, 7, and 14 after the test article administration. No deaths or notable changes in health were observed in either male and female rats. Normal body weight gains were observed, with no notable histopathology findings indicated at the end of the 14-day observation period. Therefore, the LD_{50} value in the acute oral toxicity study of MLCT-Oil was found to be greater than 5000 mg/kg body weight, with no toxic effects noted at this level.

MCT have also been evaluated for acute toxicity. A summary of unpublished studies indicate that mice (Tyler's Original strain, number of mice *per* group was not provided) were treated (*via* gavage) with 5.0, 10.0, 20.0, and 25.0 ml/kg bw of MCT (Miglyol 812®, a solution containing caprylic and capric triglycerides) in a range-finding test (doses approximately

equivalent to 4.59, 9.19, 18.38, and 22.98 g/kg, respectively), in which no deaths were reported (Traul *et al.*, 2000). In the main study conducted with 25 ml/kg (equivalent to 22.98 g/kg), lethargy and ataxia occurred within ten minutes after oral administration of 22.98 g/kg bw, and dyspnea was noted within the first hour (group size was not indicated). All animals were asymptomatic at the end of the first day. No necropsy observations were reported. An additional mouse study evaluated MCT at 12.5, 20.0, and 25.0 ml/kg (approximately equivalent to 11.49, 18.38, and 22.98 g/kg bw, respectively). The mid- and high-dose groups experienced transient ataxia, lethargy, dyspnea and diuresis within 15 minutes of dosing, and complete loss of activity was observed within two hours, followed by recovery (no further details were provided). Deaths occurred within 24 to 48 hours in two animals in the 18.38 g/kg dose group, and one animal that received 22.98 g MCT/kg. By the end of Day 3 all symptoms disappeared in the survivors (Traul *et al.*, 2000). Transient toxicity observed in mice may be specific to that species, as a summary of an unpublished study that evaluated the acute oral toxicity of MCT in Wistar male rats administered 4.5 to 36 ml/kg MCT (equivalent to 4.14 to 33.09 g/kg MCT, respectively) reported that no toxic effect was observed during the 10-day observation period or at necropsy. The animals administered 16.54 or 33.09 g MCT/kg were noted to consume less feed and excreted softer feces for the first two days (Traul *et al.*, 2000). Additional research noted that MCT consumption enhances cholecystokinin (CCK) secretion, a known satiety hormone, explaining in part the decreased consumption of MCT (Furuse *et al.*, 1992).

6.2. Subchronic Studies

Caprenin, a randomized triglyceride comprised of caprylic (C8:0), capric (C10:0), and behenic (C22:0) fatty acids, is similar to MLCT-Oil in that caprenin contains both medium- and long-chain fatty acids on the same glycerol backbone. Webb *et al.* (1993) evaluated the safety of caprenin when fed to Sprague Dawley rats at dose levels of 5.23, 10.23, or 15.00% (w/w) for 91 days (approximately equivalent to 5230, 10,230, and 15,000 mg/kg/day, respectively). Corn oil was added at 8.96, 5.91, and 3.00%, respectively, to provide essential fatty acids and digestible fat calories. A blend of MCT oil and corn oil (11.21 and 3.13%, respectively), and corn oil alone (12.14%) served as controls. The test and control diets were formulated to provide approximately 4000 kcal/kg, with 26.8% of digestible calories coming from fat, assuming corn oil, MCT oil, and caprenin provided 9, 7, and 5 kcal/g, respectively. The diets were fed for 91 days. Survival, body weight, feed consumption, clinical signs, feed efficiency, organ weights, organ-to-body-

weight ratios, organ-to-brain weight ratios, clinical chemistry and hematological values were evaluated in all dose groups. Tissue histopathology was evaluated in the corn oil and MCT oil control groups, as well as the high-dose caprenin group. No significant differences in body weight gain were measured, although feed conversion efficiency was reduced in the high-dose caprenin group. No adverse effects were noted after ingestion of caprenin. The authors established a no-observable-adverse-effect level (NOAEL) of greater than 15% (w/w) caprenin in the diet. This equated to more than 83% of the total dietary fat, and was equal to a mean exposure level of greater than 13,200 mg/kg bw/day for male rats and greater than 14,600 mg/kg/day for female rats. This study indicates that subchronic consumption of high levels of oil containing medium- and long-chain fatty acids did not result in toxic effects in the rats.

Utilizing an enzymatically modified soybean oil with caprylic acid (8:0), Lee *et al.* (2000) found that the different positional distribution of MCFA in MLCT molecules lead to different metabolic fates, when compared to a physical mixture of tricaprylin and soybean oil (PHY), or soybean oil (SO) alone [fat consumption at 20% (approximately 20,000 mg/kg/day) of the diet for 21 days]. Female Zucker rats were fed one of three test oils, (1) MLCT, (2) PHY, or (3) SO, for 21 days, after which it was noted that caprylic fatty acids were located in the livers of MLCT-fed rats, but not in PHY-fed rats. Plasma total cholesterol and plasma triacylglycerol were significantly higher in the MLCT and PHY-fed groups, but not in the SO control group. This is in contrast to earlier work by Lee *et al.* (1999), who indicated that consumption of MLCT (containing *n*-3 polyunsaturated fatty acids and caprylic acid) fed to 4-6 week old female mice for 21 days resulted in significant decreases in total cholesterol, LDL cholesterol, and triacylglycerol, a possible species-specific effect.

Takeuchi *et al.* (2001) evaluated the effect of six-week administration of MLCT on body fat accumulation in rats. Male Wistar rats (n = 16) were randomized into two groups and fed a purified high fat diet, containing either 25% soybean oil (LCT) or MLCT (approximately equivalent to 25,000 mg/kg bw/day). Soybean oil contains virtually 100% LCT, while the MLCT in this study contained approximately 80% LCT and 20% MCT, as indicated in Table 10. Each group was given free access to the experimental diet and water for six weeks. Feces were collected for the last two days of feeding. The rats were killed by decapitation at the end of the study, with perirenal and mesenteric adipose tissue, epididymal adipose tissue, head, tail,

digestive tract, lungs, kidneys, and testes removed for analysis. The initial body weight, final body weight and six-week food intake did not differ between the two groups. The carcass fat content, perirenal and mesenteric adipose tissue weights were significantly lower in the MLCT diet group, than in the LCT group ($P<0.05$). The carcass weight and carcass protein content were not affected by MLCT administration. In addition, the liver weight and liver triacylglycerol content did not significantly differ between the two diet groups. The feces weight and digestibility of dietary fat did not significantly differ between the two diet groups. No adverse effects were noted in either dose group. This study indicates that a high-fat diet of approximately 25,000 mg/kg bw/day MLCT for six weeks is well tolerated.

Table 10. Triacylglycerol and fatty acid composition of the test oils (Takeuchi *et al.*, 2001)

	Test oil	
	Soybean oil (LCT)	MLCT
Triacylglycerol composition	(g/100g)	
L, L, L	100	38.4
L, L, M	ND	44.2
L, M, M	ND	15.9
M, M, M	ND	1.5
Fatty acid composition	(g/100g)	
8:0	ND	14.4
10:0	ND	4.8
16:0	10.5	3.2
18:0	3.8	1.6
18:1	23.6	49.2
18:2	54.2	17.9
18:3	7.6	8.9
Unidentified	0.3	ND

L=long-chain fatty acids; LCT=Long chain triacylglycerols; M=medium-chain fatty acids; MLCT=medium- and long-chain triacylglycerol; ND=Not detected

Matsuo and Takeuchi (2004) studied the effect of diets containing 5-20% MLCT on body fat accumulation, when compared to ingestion of LCT. In a rat model, Wistar rats (n = 48, four weeks old) were randomly assigned to eight groups. Four of the groups were fed diets containing 50, 100, 150, or 200 g/kg bw LCT, with the other four groups receiving 50, 100, 150, or 200 g/kg bw MLCT, respectively. These levels are comparable to 5, 10, 15, and 20% fat in the diet, or 5000, 10,000, 15,000, and 20,000 mg/kg bw *per* day, respectively. Each group of rats was given the respective experimental diet and water *ad libitum* for eight weeks. At the end of the study, the rats were killed by decapitation, blood was collected to obtain serum, with the liver and intra-

abdominal adipose (epididymal, perirenal and mesenteric) tissues removed, weighed and stored at –40°C until analysis. Serum glucose, triacylglycerols, and insulin levels were determined, as well as liver total lipids, carcass fat, carcass protein, and hepatic capacities of citrate synthase and cytochrome oxidase were measured (Matsuo and Takeuchi, 2004).

No adverse effects were noted in this study. Final body weight and weight gain were significantly greater in rats fed 15 and 20% LCT diet, when compared to those fed 5 and 10% LCT in the diet ($P<0.05$). In contrast, although the rats fed MLCT significantly increased their final body weight, the body weight gain did not vary between the groups fed MLCT, with the final body weight and body weight gain significantly lower in the 15 and 20% MLCT dose groups, when compared with the 15 and 20% LCT dose groups, respectively. In the 15 and 20% MLCT diet groups, food efficiency was significantly lower ($P<0.05$). Liver weight and triacylglycerol content were not influenced by the levels of LCT and MLCT, but liver triacylglycerol content was significantly increased in rats fed the MLCT diet, when compared to rats fed the 20% LCT/kg diet. Perirenal adipose tissue weight and *intra*-abdominal adipose tissue weight was significantly ($P<0.05$) lower in the 15 and 20% MLCT dose groups, when compared to the 15 and 20% LCT dose groups. In the 15 and 20% fat diet groups, carcass weight and carcass fat content were significantly lower ($P<0.05$) in MLCT-fed rats, when compared to LCT-fed rats. Carcass protein content was not affected by dietary fat level or composition. Serum triacylglycerol concentration was significantly higher ($P<0.05$) in MLCT-fed rats, when compared to LCT-fed rats. Serum glucose and insulin concentrations were significantly lower ($P<0.05$) in the 50 g fat/kg diet group than in 100, 150, and 200 g fat/kg diet groups in rats fed LCT and MLCT diets, but the MLCT groups were not significantly different from the corresponding LCT dose groups. In addition, the hepatic capacities of the citrate synthase and cytochrome oxidase were significantly higher ($P<0.05$) in rats fed MLCT, when compared to LCT-fed rats. This study indicates that MLCT fed at doses up to 20% (approximately 200 g/kg bw/day) of the diet was not toxic to rats, and although rats fed MLCT gained significant amounts of body fat, their gain was significantly less than rats fed a diet containing up to 20% LCT.

In a short-term study in hamsters, the effects of a two-week diet containing 10% MLCT were evaluated on potential changes in lipidemia, liver and aortic cholesterol, and fecal neutral sterol excretion (Wilson *et al.*, 2006). Male Golden Syrian hamsters were initially fed a

hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol. After two weeks on the HCD, the hamsters were divided into three groups (n = 8 *per* group) and fed either the HCD, or one of two MLCT oils: 1(3),2-dicaproyl-2(1)oleolgylcerol (OCC), or 1,3-dicaproyl-2-oleoylglycerol (COC) at 10% of the diet (approximately 10,000 mg/kg bw/day). The two MLCT oils differed only in where the oleic fatty acid was attached to the glycerol backbone. After an additional two weeks on the HCD or MLCT diets, the hamsters were euthanized and evaluated. The hamsters survived all dietary treatments, with no differences among the groups in final body weight, liver weight, or liver index (liver weight/body weight ratio). Plasma total cholesterol, high-density lipoprotein-C (HDL) cholesterol levels, and non-HDL cholesterol (a combination of very-low-density lipoproteins, intermediate, and low-density lipoprotein-C), as well as aortic total and free cholesterol levels, were reduced in hamsters fed the OCC and COC diets, compared to the HCD (P<0.05). Fecal cholesterol levels did not vary between groups. In addition, the OCC and COC groups had significantly lower plasma non-HDL/HDL cholesterol ratios, compared to the HCD (P<0.05), indicating that the MLCT oils lowered blood cholesterol levels, compared to control fed hamsters, without adverse effects (Wilson *et al.*, 2006).

In a study evaluating the safe consumption of MLCT-Oil, six-week-old male Wistar rats were individually housed, divided into groups of 20 rats *per* group, and assigned to an isocaloric diet containing 7% of either LCT (rapeseed oil) as a control, or MLCT-Oil, for six weeks (Matulka *et al.*, 2006). The *percentage* of oil contained in the diet was calculated to be approximately equal to a daily consumption at 3500 mg/kg/day LCT or MLCT-Oil, respectively. The components of the control and experimental diets are given in Table 11.

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Table 11. Components of MLCT-Oil subchronic study experimental diet (Matulka *et al.*, 2006)

Components	Amount (g/kg diet)
Cornstarch	529
Casein	200
Sucrose	100
Test oils*	70
Cellulose	50
Mineral mix	35
Vitamin mix	10
L-Cysteine	3.0
Choline bitartrate	2.5
tert-Butylhydroquine	0.014

*Test oil for the control diet was rapeseed oil. The study test oil was MLCT-Oil; MLCT=medium- and long-chain triacylglycerol

Following administration of the study diet for six weeks, the rats were anesthetized with diethyl ether and euthanized *via* decapitation. Blood was collected and serum separated and stored at -80°C until analysis. Weights were determined for the adipose tissue surrounding the epididymis, the kidneys, and the mesenteric adipose tissue. In addition, liver weight, subcutaneous fat (minus abdominal fat) weight and carcass protein were determined for each rat.

Serum neutral fats and total cholesterol concentration were analyzed enzymatically using a Hitachi 7170 automatic analyzer. For the analysis of subcutaneous fat weight and carcass protein amount, the carcasses were freeze-dried and uniformly pulverized. Subcutaneous fat was determined as the amount obtained by extraction with diethyl ether. Body protein weight was performed *via* determination of the total nitrogen, and a nitrogen-protein conversion coefficient of 6.25 was utilized in the protein determinations.

Good nutritional status, as indicated by a lack of difference between the control and MLCT-Oil body weight changes, was achieved over the six-week period (Table 12). Compared to the control group, the MLCT-Oil treated rats (at approximately 3500 mg/kg/day MLCT-Oil) demonstrated a significantly ($P<0.05$) increased food and caloric consumption during the six-week study period, although body fat and body fat ratio (as grams body fat/energy intake) were significantly less than controls. The MLCT-Oil group also had significant increases in total carcass protein amounts and significantly lower serum cholesterol values. Triacylglycerol values were not significantly different from controls. No adverse effects were noted. No other

significant differences between groups were noted. The no observed adverse effect level (NOAEL) in this study was 3500 mg/kg/day MLCT-Oil (Matulka *et al.*, 2006).

Table 12. Effect of MLCT-Oil on rat carcass protein, food and energy intake, body fat, and serum lipids (Matulka *et al.*, 2006)

	Experimental Diets	
	Control (LCT)	MLCT-Oil
Initial bodyweight (g)	167.2±1.4	167.2±1.4
Final bodyweight (g)	287.1±2.7	291.2±2.5
Weight gain (g/6 weeks)	119.9±2.4	124.0±1.8
Food intake (g/6 weeks)	547.2±5.0	573.1±3.0*
Energy intake (kcal/6 weeks)	2178.4±20.0	2276.1±11.9*
Carcass protein (g/100g bodyweight)	14.8±0.1	15.3±0.1*
Body fat (g)	35.9±0.9	31.8±0.9*
Body fat (g/energy intake: kcal 6 weeks)	16.5±0.3	14.0±0.4*
Triacylglycerol (mg/dl)	78.5±5.9	69.3±6.5
Total cholesterol (mg/dl)	63.9±1.6	56.0±1.4*

Values are means ± SEM, n = 20 rats/group. *Significantly different at $P<0.05$; LCT=Long-chain triglycerols; MLCT=medium- and long-chain triacylglycerol

Nisshin OilliO, Ltd. also submitted a summary of an unpublished subchronic safety study that evaluated the effects of ingestion of MLCT-Oil by rats for 13 weeks. Male and female Sprague Dawley rats (n = 10 *per* sex *per* group) were administered MLCT-Oil (500 or 2000 mg/kg bw/day) or corn oil (control) for 91 days, with general observations for potential toxicity conducted before and after test article administration. Body weight and food consumption was measured once a week during the study, and immediately prior to euthanization. Urinalysis (bilirubin, glucose, ketone body, occult blood, pH, protein, urinary resident, and urobilinogen) was performed on fresh and 24-hour samples on Day 87 for males and Day 89 for females. Blood analysis (activated partial thromboplastin time, fibrinogen time, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, prothrombin time, platelet, red blood cell, and white blood cell levels) and hematologic biochemical tests (ALP, ALT, AST, creatinine, glucose, total cholesterol, triglyceride, total protein, urea nitrogen, total bilirubin, inorganic phosphorus, calcium, sodium, potassium, chloride, and protein fractions) were analyzed from arterial blood obtained from the aorta abdominalis the day after the last test article administration. Histological observations were performed on the adrenal glands, brain, heart, kidneys, liver, lungs, male and female sex organs, pituitary glands, salivary glands, spleen, thymus, and thyroid glands.

No deaths were observed in any of the treatment groups during the 13 week study. No significant changes were noted in any of the blood chemistries or histological sections.

6.3. Genotoxicity Studies

The potential mutagenic effect of MLCT was assessed by exposing four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and one strain of *Escherichia coli* (WP2*uvrA*) to five different concentrations of the test material, based on previously published *in vitro* techniques (Ames *et al.*, 1973; McCann *et al.*, 1975; Matulka *et al.*, 2006). It is known that some chemicals do not exert a mutagenic effect in this system unless they are activated by mammalian enzymes. The metabolic activation is accomplished by incubating the bacteria together with the test compound and enzyme mix, consisting of rat liver (Sprague Dawley rat livers induced by *in vivo* administration of phenobarbital or 5,6-benzoflavone) enzymes supplemented with salts and co-factors (S9). Two independent genotoxicity tests were performed, utilizing all five bacterial strains, with and without S9. A solvent treatment group was used as the negative control, with the positive controls treated as follows: 2-(2-furyl)-2-(5-nitro-2-furyl)acrylamide (AF-2), 0.1 µg/plate for TA98; 0.01 µg/plate for TA100 and WP2*uvrA*; sodium azide (NaN_3), 0.5 µg/plate for TA1535; 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]-acridine 2HCl (ICR-191), 1.0 µg/plate for TA1537. In addition, for the S9 series of plates, the materials, benzo(a)pyrene [B(a)P], 5 µg/plate for TA98, TA100, and TA1537 and 2-aminoanthracene (2AA), 2 µg/plate for TA1535 and 10 µg/plate for WP2*uvrA* were used as positive controls.

In a concentration range-finding test, MLCT oil did not induce mutations, with or without metabolic activation, in any of the tested strains, and did not inhibit the growth of any of the strains up to 5000 µg/plate (Matulka *et al.*, 2006). Based on the results of the range-finding test, the highest dose level in the main test was set at 5000 µg/plate for all tester strains, with or without metabolic activation. A total of five dose levels were included in the main test, utilizing four stepwise dilutions with a common ratio of 2 (*i.e.*, 313, 625, 1250, 2500, and 5000 µg/plate). The positive controls increased the number of revertant colonies more than twice that of the vehicle control of each tester strain (Table 13). The number of revertant colonies in each tester strain in the vehicle and positive control groups (with or without metabolic activation) was comparable with that in the historic data of the testing facility (data not shown). Administration

of MLCT oil did not result in any concentration-related increases in revertant colonies/plate in either the *S. typhimurium* strains or the *E. coli* strain, with or without exogenous metabolic activation, and hence was deemed non-genotoxic (Table 13) (Matulka *et al.*, 2006).

Table 13. Number of bacterial revertants (number of colonies/plate) after incubation with MLCT (Matulka *et al.*, 2006)

	Dose (µg/plate)						
Strain	0	313	625	1250	2500	5000	Positive Control
(-) TA98#	24	25	17	17	22	4	501
(-) TA100*	129	140	123	136	122	36	410
(-) TA1535*	21	29	19	23	23	0	455
(-) TA1537#	15	13	14	12	16	8	1528
(-)WP2uvrA*	23	19	27	22	22	4	168
(+) TA98#	33	46	35	33	45	2	273
(+)TA100*	143	140	137	141	130	56	1123
(+)TA1535*	20	23	21	17	23	0	356
(+) TA1537#	27	25	21	22	24	5	126
(+)WP2uvrA*	38	33	39	35	38	5	731

(-) = bacteria and MLCT incubated in absence of S9 mix; (+) = bacteria and MLCT incubated in presence of S9 mix; * = Base-pair substitution type mutation; # = Frameshift type mutation ; MLCT=medium- and long-chain triacylglycerol

7. CLINICAL STUDIES

Swift *et al.* (1992) assessed fasting plasma lipids, lipoproteins, and postprandial plasma lipids in a randomized, placebo-controlled crossover study in healthy male subjects who consumed a structured medium- and long-chain triglyceride. The structured triglyceride (caprenin) consisted mostly of C8:0, C10:0, and C22:0 fatty acids. Ten healthy males were fed a liquid-formula diet containing 40% of total energy as LCT, MCT, or caprenin, for six days. The subjects received a 4.18 MJ (1000-kcal) test meal containing the appropriate lipid substance. The caprenin diet consisted of 20% soybean oil and 80% caprenin (equivalent to ingestion of 35.5 g caprenin/day). Total blood serum cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides, and plasma lipid concentrations were determined prior to study initialization, and on Day 6 of the study. None of the diets altered plasma cholesterol concentrations. HDL cholesterol was decreased 14% by the caprenin diet ($P<0.05$) and 15% by the MCT diet ($P<0.005$), but was unchanged by the LCT diet. Plasma triglycerides were elevated 42% by the

MCT diet ($P<0.01$), but unaltered in the other diets. This study indicates that short-term feeding of MCT or caprenin diets produced significant lipid metabolism increases (Swift *et al.*, 1992).

Wardlaw *et al.* (1995) utilized caprenin in randomized, blinded studies to measure changes in serum cholesterol, and other serum lipids, and apolipoprotein concentrations in hypercholesterolemic men consuming caprenin-rich diets. Hypercholesterolemic men (n = 17) who consumed a caprenin-rich diet (up to 71 g caprenin/day) showed significant reductions in HDL cholesterol, and a significant increase in the total cholesterol:HDL ratio. No significant changes in the lipid and apolipoprotein indices were noted. The authors concluded that one or more of the 8:0, 10:0, and 22:0 fatty acids in the caprenin diet could contribute to hypercholesterolemia (Wardlaw *et al.*, 1995).

Early work analyzed the effects of a structured lipid emulsion containing medium- and long-chain fatty acids randomly esterified to a glycerol backbone in a triglyceride structure, when compared to MCT or LCT. Sandstrom *et al.* (1995) evaluated the rate of oxidation of structured medium- and long-chain fatty acid lipids in postoperative patients, when compared to patients infused with LCT alone. The study design was conducted in two parts. In the first part, one-half of the total 19 patients (13 male/6 female, aged 22-79 years) were randomized to receive structured triglycerides on postoperative days 1, 3, and 5, and LCT on postoperative days 2, 4, and 6. In the second part, the patients received LCT on postoperative days 1, 3, and 5, and structured medium- and long-chain fatty acid lipids on postoperative days 2, 4, and 6. The infusions started on the day after the operation and continued for six days. All infusions were administered *via* a central venous catheter. In part 1, structured triglycerides and LCT were infused at 0.01 ml/kg/minute for a total of 1.0 g/kg/day lipid. In part 2, lipids were provided at a total of 1.5 g/kg/day. Laboratory tests included plasma electrolytes, kidney and liver function tests, serum protein, and creatinine concentrations.

There were no signs of allergic reactions, central nervous system effects, nausea, or chills. Biochemical tests and clinical variables (*e.g.*, blood pressure, respiratory frequency, heart rate, body temperature) did not show any differences during infusion with any of the two lipid emulsions in either part 1 or part 2 of the study. Plasma triglycerides, free fatty acids, glycerol, and 3-hydroxybutyrate concentrations were not different between the two lipid emulsions. In part

2, structured lipids caused a significantly higher plasma concentration of glycerol, free fatty acids, and 3-hydroxybutyric acid, compared with LCT. Respiratory gas exchanges and derived metabolic rates did not differ between the lipid emulsions. Infusion of structured lipids was associated with a trend to a higher whole body fat oxidation rat ($P<0.1$) in part 1, while a greater amount of structured lipids given in part 2 of the study resulted in a significant increase in whole body fat oxidation ($P<0.0001$). This study indicates that structured triglycerides, which are similar to MLCT, increase whole body fat oxidation in stressed postoperative patients (Sandstrom *et al.*, 1995).

The effects of a liquid-formula diet supplement containing structured medium-and long-chain triacylglycerols (SMLCT), composed of 10% medium and 90% long-chain fatty acids, were compared with ingestion of only long-chain fatty acid triacylglycerols (LCT, soybean oil) for body fat accumulation in a clinical trial (Matsuo *et al.*, 2001a). Thirteen healthy male volunteers, aged 18-20 years, were randomly assigned the SMLCT ($n = 7$) or LCT ($n = 6$) group. The subjects in each group received a liquid-formula diet supplement containing 1040 kJ (248.56 kcal) plus daily energy intake, from either SMLCT or LCT (20 g/day), in addition to their typical food intake. The composition of fatty acids and triacylglycerol of the test oils are presented in Table 14. Body compositions were measured weekly, from which the body mass index (BMI) was calculated. In addition, the *percentage* of body fat, fat mass and fat-free mass were determined, and the waist-to-hip ratio was calculated.

At the end of the 12-week study, the bodyweight of male subjects in both groups increased slightly, but not significantly from baseline. All groups showed significant increases in body fat *percentages* from baseline throughout the 12-week period. No adverse effects were noted during the 12-week study. Rates of variation of body fat *percentages* were significantly lower in the SMLCT group than the LCT controls ($P<0.05$). Increases in body fat *percentages* at the end of the study were 2.2 and 4.3% for the SMLCT and LCT groups, respectively, with the increases by the LCT groups that were significantly greater than that of the SMLCT groups. Fat weight gains over the 12-week study were 2.01 and 2.79 kg for the SMLCT and LCT groups, respectively. The biochemical parameters of blood, plasma and serum did not vary between

groups.[13] In addition, the change in fat free mass did not differ between groups. These results indicate that SMLCT ingestion, at a dose of 20 g *per* day for twelve weeks, which provided 1040 kJ plus daily energy intake, resulted in less body fat accumulation than those receiving 20 g LCT for twelve weeks, while maintaining fat free body mass (Matsuo *et al.*, 2001a).

Table 14. Triacylglycerol and fatty acid composition of test oils administration to male subjects (Matsuo *et al.*, 2001a).

	Test oil	
	Soybean oil (LCT)	SMLCT
Triacylglycerol composition	(g/100g)	
L, L, L	100	63.3
L, L, M	ND	28.9
L, M, M	ND	6.4
M, M, M	ND	1.4
Fatty acid composition	(g/100g)	
8:0	ND	7.3
10:0	ND	2.4
16:0	10.4	3.8
16:1	0.1	0.2
18:0	4.0	1.9
18:1	23.9	55.2
18:2	52.9	18.3
18:3	7.8	7.7
20:0	0.3	0.6
20:1	0.2	1.4
22:0	0.4	0.3
22:1	ND	0.5
24:0	ND	0.2
24:1	ND	0.2
Total	100.0	100.0

L=long-chain fatty acids; LCT=Long-chain triacylglycerols; M=medium-chain fatty acids; SMLCT=structured medium- and long-chain triacylglycerol; ND=Not detected

Matsuo *et al.* (2001b) also evaluated the effect of structured MLCT (SMLCT) on the basal metabolic rate, when compared to LCT (*i.e.*, soybean oil), in young women in a randomized crossover clinical trial. Fifteen young women (aged 18-28 years) who did not customarily exercise were randomized into two groups and orally administered an acute dose (1680 kJ test oil) of either LCT or SMLT (approximately equivalent to 43 g). The triacylglycerol

[13] Parameters included glucose, insulin, triacylglycerol, free fatty acids, total cholesterol, HDL-cholesterol, acetoacetic acid, 2-hydroxybutylate, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells, and platelets.

and fatty acid composition of the test oils are presented in Table 15. The subjects then rested for six hours, during which the oxygen consumption and nonprotein respiratory quotient (RQ) were measured by indirect calorimetry. Blood samples were obtained at 0, 1, 2, 3, 4, and 6 hours post ingestion.

Table 15. Triacylglycerol and fatty acid composition of test oils to female subjects (Matsuo *et al.*, 2001b)

	Test oil	
	Soybean oil (LCT)	SMLCT
Triacylglycerol composition	(g/100g)	
L, L, L	100	38.4
L, L, M	ND	44.2
L, M, M	ND	15.9
M, M, M	ND	1.5
Fatty acid composition	(g/100g)	
8:0	ND	13.7
10:0	ND	4.7
16:0	10.4	3.6
16:1	0.1	0.2
18:0	4.0	1.8
18:1	23.9	50.1
18:2	52.9	16.1
18:3	7.8	7.4
20:0	0.3	0.5
20:1	0.2	1.1
22:0	0.4	0.3
22:1	ND	0.3
24:0	ND	0.1
24:1	ND	0.1
Total	100.0	100.0

L=long-chain fatty acids; LCT=Long-chain triacylglycerols; M=medium-chain fatty acids; SMLCT=structured medium-and long-chain triacylglycerol; ND=Not detected

Post-ingestive total energy expenditure (PTEE) increased significantly in female subjects after SMLCT administration, when compared with LCT ingestion (26.9±1 *vs.* 25.5±1.1 kJ/kg/6 hr, $P<0.05$), respectively. The thermic effect of the test oil was also significantly higher after SMLCT ingestion, compared with LCT ingestion (3.02±0.49, *vs.* 1.47±0.82 kJ/kg/6 hr, $P<0.01$). Oxygen consumption tended to be greater after SMLCT consumption than for LCT consumption, but significance was only reached at the 120-minute time point after SMLCT ingestion ($P<0.05$), when compared to LCT ingestion. Plasma glucose concentrations were not significantly different between groups, but the serum insulinemic response to SMLCT ingestion

at 120 minutes was significantly higher than during LCT ingestion ($P<0.05$). In addition, glycerol concentrations were significantly lower ($P<0.05$) with SMLCT administration at 60, 120, and 150 minutes post-dose. Serum free fatty acid and 3-hydroxybutyrate concentrations were significantly increased after SMLCT ingestion, when compared to LCT ingestion, at 120 and between 60 to 240 minutes, respectively. These results indicate that consumption of SMLCT may increase the resting metabolic rate over six hours (Matsuo *et al.*, 2001b).

Takeuchi *et al.* (2002) administered MLCT to college athletes in a double blind crossover study, in which for three weeks male subjects (n = 6, aged 20±1 years) ingested a liquid diet containing 20 g/day of soybean oil (LCT) or MLCT, in addition to their regular diet. The triacylglycerol and fatty acid compositions of the test oils are presented in Table 16. Blood samples were taken at 0, 2, and 3 weeks of the treatment period. Serum triacylglycerol, cholesterol, insulin, acetoacetic acid, 3-hydroxybutyric acid, total ketone bodies, nonesterified fatty acids, and glycerol, as well as plasma glucose, were evaluated. No adverse reactions were noted. The supplementation with either MLCT or LCT had no effect on body weight or body mass index. The MLCT-supplemented diet maintained serum triacylglycerol levels in the subjects, while the LCT diet significantly increased ($P<0.05$) the rate of variation (*i.e.*, change over time of the measurements when compared to week 0) of serum triacylglycerol levels. Serum cholesterol or insulin levels did not differ between the MLCT- or LCT-supplemented subjects. After three weeks of consuming the MLCT diet, the rate of variation of body-fat mass was significantly lower than after the LCT diet period ($P<0.05$). This study indicates that energy derived from MLCT is less likely to accumulate into human adipose tissue than energy derived from soybean oil.

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Table 16. Control and test oil triacylglycerol and fatty acid composition (*Takeuchi et al.*, 2002)

	Test oil	
	Soybean oil (LCT)	MLCT-Oil
Triacylglycerol composition	(g/100g)	
L, L, L	100	38.4
L, L, M	ND	44.2
L, M, M	ND	15.9
M, M, M	ND	1.5
Fatty acid composition	(g/100g)	
8:0	ND	14.4
10:0	ND	4.8
16:0	10.5	3.2
18:0	3.8	1.6
18:1	23.6	49.2
18:2	54.2	17.9
18:3	7.6	8.9
Others	0.3	--
Total	100.0	100.0

L=long-chain fatty acids; LCT=Long-chain triacylglycerol; M=medium-chain fatty acids; MLCT=medium- and long-chain triacylglycerol; ND=Not detected

Kasai *et al.* (2003) investigated whether a structured medium- and long-chain triacylglycerol (MLCT) diet could decrease body fat accumulation in healthy subjects. A double blind, placebo-controlled randomized study followed the daily consumption of test bread made with 14 g of MLCT containing 1.7 g MCFA in healthy subjects (n = 40, 36 men and 4 women) for twelve weeks, and compared this with control subjects (n = 42, 39 men and 3 women) that consumed bread made with long-chain triacylglycerols (LCT). All subjects consumed the same standard packaged meals throughout the 12-week period. Outcome parameters included body weight, total body fat and abdominal fat, body mass index (BMI), serum cholesterol, triacylglycerols and phospholipids. The baseline data for energy and fat intake during the study was determined from a 3-day preliminary investigation conducted prior to the study.

There were no differences in energy intake, major nutrient intake, dietary fat composition, dietary cholesterol and alcohol intakes between the MLCT and LCT groups. During the 12-week study, the MLCT group ingested significantly higher (P<0.001) levels of medium-chain fatty acids than the LCT group. The intake of (n-6) polyunsaturated fatty acids (PUFA) in the LCT group was significantly higher than the MLCT group (P<0.001). There were no other significant differences in nutrient or dietary lipid composition intakes during the study

period. Body weight and BMI at four, eight, and twelve weeks decreased significantly among both groups ($P<0.001$), with the overall *percent* of BMI and bodyweight decrease significantly greater in the MLCT group, as compared to the LCT group ($P<0.05$). A significant *percent* of reduction in body fat was noted in the MLCT group, as compared to the LCT group at both 4 and 8 weeks into the study ($P<0.01$ and $P<0.05$, respectively), but the significance from the LCT group was not maintained at the twelve-week time point. In addition, the total fat area was significantly less in the MLCT group at eight and twelve weeks, when compared to the LCT group ($P<0.01$).[14] The only alteration in the blood chemistry values was a significant decrease in serum total cholesterol in the MLCT group at eight weeks, when compared to the LCT group at the same time point ($P<0.05$). Changes in serum HDL cholesterol concentrations did not differ significantly between groups, although the decrease in serum LDL cholesterol in the MLCT group was greater than that of the LCT group. No adverse reactions were noted during the study period. This study indicates that MLCT, when consumed as part of a food product, may be safely consumed at up to 14 g *per* day for twelve weeks (Kasai *et al.*, 2003).

Matulka *et al.* (2006) reported the effects of MLCT-Oil dietary consumption on serum lipids, ketone bodies, body fat, liver function, and renal function in humans. Ten male and female subjects consumed either LCT (*e.g.*, vegetable oils) at 14 g *per* day, or 42 g MLCT-Oil *per* day in a placebo-controlled, double blind randomized, controlled four-week study. The participants were controlled for the caloric energy-to-fat consumption amounts and BMI. The test subjects were healthy Japanese men and women (ages 21-39 years) and the study excluded individuals with diabetes, hypertension or hyperlipidemia. Preliminary tests determined height, weight, normal caloric energy and fat consumption (calculated from a three-day food intake survey).

The study food (bread) containing either LCT (rapeseed oil control) or MLCT was consumed during the morning, afternoon, and evening meals. The amount of MLCT-Oil consumed *via* the bread source was calculated at 42 g *per* day. Food consumption was targeted at an average daily intake of 2100-2600 kcal and 70-80 g of fat. Prior to the study, food menu guidance was performed for individual circumstances, so that the caloric energy and fat intake consumption for one day on the control diet was within a range of the targeted caloric

[14] The subcutaneous and visceral fat areas were determined from computed tomography (CT) images at the umbilical level (Kasai *et al.*, 2003).

consumption when consuming the test (MLCT) food product. Intake of MCFA from other sources was determined in all participants. Finally, alcoholic beverages were limited to an ethanol equivalent of 25 ml *per* day. Each subject recorded the daily consumption of food (including the study food), side dishes, and beverages.

Body measurements were conducted during a fasting state and, body weight, waist (circumference measurement at the umbilical point), hip (measured as a maximum circumference), and body fat ratio (air value substitution method) were recorded. Blood and urine samples were collected at study initialization and study completion, during a fasting state (excluding water consumption). Urinary protein, blood sugar, ketone bodies, urobilinogen, occult blood, pH, serum cholesterol, neutral fats, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), were determined. Hematology parameters, as well as insulin, and lipid peroxide were also analyzed. Statistical significance was determined for the average ± standard deviation, with the two-way analysis of variance assay and Mann-Whitney-U tests. A P value of less than 5% was determined as significant.

MCFA consumption was significantly different ($P<0.05$) between the LCT (*i.e.*, mixed rapeseed and soybean oils (7:3) as a control) and MLCT groups (LCT food group consumed 0.1 g MCFA/day; MLCT food group consumed 5.1 g MCFA/day, respectively). However, the caloric energy and fat consumption were 2200 kcal and 73 g, respectively, and not significantly different between groups (Table 17).

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Table 17. Nutritional consumption *per* day during the four-week clinical trial (Matulka *et al.*, 2006)

	Experimental Diets	
	Control (LCT)	MLCT-Oil
Energy intake (kcal/day)	2166±85	2213±135
Fats (kcal/day)	72.5±1.1	73.3±1.3
Protein (kcal/day)	67.2±4.5	68.2±5.6
Carbohydrate (kcal/day)	301±15	317±36
Medium chain fatty acid (g/day)	0.1±0.0	5.1±0.0*
Cholesterol (mg/day)	304±20	308±24

Values are means ±S.D.; *Significantly different at $P<0.05$; LCT=Long-chain triacylglycerols; MLCT=medium- and long-chain triacylglycerol

Serum cholesterol was not significantly different between the LCT and MLCT groups after the four-week study. Compared with study initiation values (LCT; 182±22 mg/dL, MLCT; 175±29 mg/dL), the serum cholesterol had nonsignificantly decreased after the four-week study within each group (LCT; 154±20 mg/dL, MLCT; 152±33 mg/dL), respectively (Table 18). The serum LDL and HDL cholesterol also decreased after the four-week study, but significance was not reached.

Table 18. Serum cholesterol, neutral fats, and respective lipoproteins at study initiation and after Week 4 of the clinical trial (Matulka *et al.*, 2006)

Lipids (mg/dL)	Time Period	LCT Food group[a]	MLCT food group[a]
Total Cholesterol	Initiation	182±22	175±29
	After 4 Weeks	154±20	152±33
VLDL Cholesterol	Initiation	15.2±6.3	14.8±8.8
	After 4 Weeks	16.4±4.8	17.4±10.4
LDL Cholesterol	Initiation	99.3±20.3	95.0±26.8
	After 4 Weeks	79.6±20.5	79.3±27.6
HDL Cholesterol	Initiation	66.6±13.4	64.2±10.1
	After 4 Weeks	57.5±9.4	54.9±7.8
Neutral Fats (TG)	Initiation	64±23	64±31
	After 4 Weeks	59±15	63±38
VLDL TG	Initiation	29.6±20.2	31.3±24.1
	After 4 Weeks	27.9±12.7	34.1±30.0
LDL TG	Initiation	22.4±3.8	20.2±5.6
	After 4 Weeks	19.3±4.7	18.4±6.8
HDL TG	Initiation	10.1±2.1	10.0±3.2
	After 4 Weeks	9.8±2.4	10.0±2.3

HDL=high density lipoprotein; LCT=long-chain triacylglycerol; LDL=low density lipoprotein; MLCT=medium- and long-chain triacylglycerol; TG=triacylglycerol; VLDL=very low density lipoprotein; [a]Average value of 10 individuals in each group ± standard deviation (SD)

Serum triacylglycerol, VLDL, LDL, and HDL triglyceride concentrations were not significantly different between or within LCT and MLCT groups at study initiation or after the four-week period (Table 18). Likewise, there were no significant differences in liver and renal function values, urinalysis values (Table 19) or hematology assessment parameters (Table 20) between or within groups at study initiation and termination. Body measurement values indicated a slight, but nonsignificant decrease in both groups for all measurements (body weight, body mass index, waist, hip, and body fat amounts.

Table 19. Hepatic and renal assessment at study initiation and after Week 4 of the clinical trial (Matulka *et al.*, 2006)

Liver Function Tests	Time Period	LCT Food group[a]	MLCT food group[a]
GOT (IU/L)	Initiation	19±4	19±3
	After 4 Weeks	19±5	20±3
GPT (IU/L)	Initiation	14±4	16±6
	After 4 Weeks	15±6	13±7
LDH (U/L)	Initiation	168±24	168±19
	After 4 Weeks	176±23	172±27
ChE (U/L)	Initiation	301±79	310±81
	After 4 Weeks	292±72	307±81
Total Protein (g/dL)	Initiation	7.7±0.4	7.4±0.3
	After 4 Weeks	7.4±0.5	7.3±0.4
Albumin (g/dL)	Initiation	5.0±0.3	5.0±0.1
	After 4 Weeks	4.7±0.3	4.6±0.3
ALP (U/L)	Initiation	195±47	205±32
	After 4 Weeks	190±50	218±39
γ-GTP (IU/L)	Initiation	20±13	22±12
	After 4 Weeks	17±10	21±11
ZTT (U)	Initiation	5.5±2.7	5.8±2.0
	After 4 Weeks	5.3±2.6	5.4±2.1
Total Bilirubin (mg/dL)	Initiation	0.7±0.3	1.1±0.6
	After 4 Weeks	0.8±0.3	1.2±0.5
Renal Function Study			
BUN (mg/dL)	Initiation	12±3	13±3
	After 4 Weeks	12±3	13±4
Creatinine (mg/dL)	Initiation	0.7±0.2	0.8±0.1
	After 4 Weeks	0.7±0.2	0.8±0.1
Uric Acid (mg/dL)	Initiation	5.1±1.2	5.3±1.2
	After 4 Weeks	5.1±1.2	5.1±1.3

ALP= alkaline phosphatase; BUN= Blood urea nitrogen; ChE=cholinesterase; GOT= aspartate aminotransferase; GPT= alanine aminotransferase; γ-GTP= γ-glutamyltranspeptidase; LCT=long-chain triacylglycerol; LDH= lactate dehydrogenase; MLCT= medium- and long-chain triacylglycerol; ZTT= zinc turbidity test; [a]Average value of 10 individuals in each group ± standard deviation (SD)

Table 20. Blood chemistry and hematology assessment of serum markers after a four-week clinical trial of MLCT-Oil consumption (Matulka *et al.*, 2006)

Serum Assessment	LCT Food Group[a]	MLCT-Oil Food Group[a]
Sugar and Fat Metabolism Study		
Glucose (mg/dL)	84±5	85±6
Insulin (μU/mL)	4±2	6±5
Total Ketone Bodies (μmol/L)	49±18	63±43
3-Hydroxybutyric Acid (μmol/L)	40±16	51±36
Acetoacetic Acid (μmol/L)	9±3	12±7
Phospholipids (mg/dL)	179±19	177±22
Free Fatty Acids (mEq/L)	0.42±0.12	0.47±0.08
Lipoperoxides (nmol/mL)	2.2±0.3	2.3±0.5
Hematology		
Red Blood Cells (*10^4/uL)	439±56	469±39
White Blood Cells (/μL)	4680±1305	5350±1044
Platelets (*10^4/uL)	21.3±6.9	21.2±4.2
Hemoglobin (g/dL)	13.6±1.8	14.2±1.3
Hematocrit Value (%)	44.9±4.8	47.3±4.0
MCV (fl)	103±4	101±4
MCH (pg)	31.0±1.0	30.3±1.2
MCHC (%)	30.2±0.9	30.0±0.9
Serum Electrolytes		
Sodium (mEq/L)	142±2	142±1
Potassium (mEq/L)	4.1±0.4	3.8±0.3
Inorganic Phosphorous (mg/dL)	3.5±0.5	3.4±0.5
Chloral (mEq/L)	101±2	101±1
Calcium (mg/dL)	9.2±0.4	9.1±0.2

fl=femtoliter; LCT=Long-chain triacylglycerol; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; MCV=mean corpuscular volume; MLCT=medium- and long-chain triacylglycerol; pg=picogram; [a]Average value of 10 individuals in each group ± standard deviation (SD)

No significant differences were noted in serum lipids, body fat, clinical chemistry, hematology, ketone bodies, or renal and hepatic assessments between the LCT and MLCT groups after four weeks of consumption. No significant differences were noted in liver and renal function, or hematology and blood chemistry parameters tested. No adverse reactions were reported by any of the subjects during and after the study (Nisshin, 2006b). This study indicated that MLCT did not affect liver function and renal function at 42 g MLCT *per* day for four weeks in healthy subjects.

8. EVALUATION

MLCT-Oil is a cooking oil containing triacylglycerols composed of a glycerol backbone with medium- and long-chain fatty acids randomly bound to the sn1, sn2, or sn3 positions. MLCT-Oil contains mostly monounsaturated fatty acids, and nearly equal minor portions of saturated and polyunsaturated fatty acids. It contains approximately 12% MCFA (as caprylic and capric acids) and approximately 75% LCFA (as oleic, linoleic, and linolenic acids).

Conformational analysis indicates that the majority (up to 92.4%) of triacylglycerols in MLCT-Oil contain at least two long chain fatty acids. MLCT-Oil is manufactured *via* transesterification from common edible vegetable oils (the source of long-chain triacylglycerols) and medium-chain triglycerols, the latter produced from coconut and palm kernel oils. MLCT-Oil is approved for food use in Japan and in the US, with all of the constituents in MLCT-Oil either approved food ingredients (GRAS), or are normal constituents found in commonly consumed foods at similar concentrations.

Metabolism studies indicate that MLCT-Oil is readily broken down into medium- and long-chain fatty acids, and absorbed *via* the portal or lymphatic route, based on the fatty acid chain length. Medium-chain fatty acids are directly absorbed into the portal vein, preferentially oxidized in the liver, and ultimately metabolized to carbon dioxide, acetate, and ketones. Long-chain fatty acids and sn2 long-chain monoacylglycerols are packaged into micelles, which are then absorbed across the intestinal mucosa. The sn2 monoacylglycerols and long-chain fatty acids are reformed into triacylglycerols, and secreted into the lymphatic system as chylomicrons, for eventual uptake into the adipose tissues for storage and later release as an energy source.

Preclinical and clinical studies used to evaluate the safety of MLCT-Oil. MLCT-Oil was found to be non-toxic in acute and subchronic animal studies. A single oral administration of MLCT-Oil at 5000 mg/kg body weight to rats did not result in lethality or toxicity over the following two-week observation period. A six-week repeat-dose study in rats administered approximately 3500 mg/kg/day MLCT-Oil did not result in any adverse effects, with no changes in the overall weight gain over the six-week period. Structurally similar MLCT oils (containing approximately 20% MCFA and 80% LCFA) have been administered to rats at up to 25% of the diet (approximately 25,000 mg/kg bw /day) for six weeks, with no adverse effects noted. MLCT-Oil was not mutagenic in an Ames assay, at doses up to 5000 μg/plate in the absence or presence of metabolic enzymes.

The constituents of MLCT-Oil, vegetable oil (*i.e.*, LCT) and MCT, have been consumed in the human diet for years, with no toxic effects. MCT has been administered parentally in clinical settings to safely provide calories to patients that cannot adequately digest common vegetable oils. Human clinical intervention studies indicate that consumption of MLCT-Oil is

well tolerated. Clinical studies with other MLCT oils similar in structure to MLCT-Oil have been orally administered to healthy subjects at doses up to up to 20 g/day for twelve weeks, which did not result in any adverse effects. MLCT-Oil consumption at 42 g *per* day for four weeks in a double blind, randomized, controlled study that specifically analyzed serum lipids, ketone bodies, body fat *percentage*, and liver and renal functions did not result in any adverse effects.

MLCT-Oil is stable at ambient temperatures for up to twelve months, and is stable during heating to 200°C for 30 minutes. MLCT-Oil can be used in place of the edible vegetable oil conventionally used in the production of processed food. Nisshin OilliO Group, Ltd. proposes to use MLCT-Oil as a replacement for common vegetable oil, and in salad dressings, margarine spreads, and frozen dinners (see APPENDIX B). A complete replacement of the vegetable oils used in these foods will result in an estimated daily intake mean and 90th percentile consumption of 11.4 and 30.9 g MLCT-Oil/person/day, or 183.4 and 459.9 mg/kg/day, respectively.

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9. CERTIFICATION

The undersigned authors of this document—a dossier in support of GRAS status determination for food ingredient use of MLCT-Oil—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

02 Oct 06

Ray A. Matulka, Ph.D. — Date
Toxicologist
Burdock Group

2 oct '06

George A. Burdock, Ph.D. — Date
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
President, Burdock Group

10. CONCLUSION

After critically evaluating the information available, the Expert Panel has determined that, based on information available throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, MLCT-Oil, in accordance with the manufacturing control standards and the quality control standards of the International Organization for Standardization (ISO), is Generally Recognized As Safe (GRAS), by scientific procedures, under the intended conditions of use, such that total daily intake of MLCT-Oil from all sources is expected to be approximately 31 g/day (*i.e.*, 517 mg/kg/day) at the 90th percentile, based on the expected daily intake when added to the food categories identified herein.

11. SIGNATURES

Robert Nicolosi, Ph.D. — Date: 6/OcT 06

I. Glenn Sipes, Ph.D. — Date: 30 Oct 06

John Thomas. Ph.D., F.A.T.S. — Date: 6 oct 06

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Nisshin (2006c) Fatty acid composition. (Personal Communication)

Nisshin (2006d) Manufacturing process for MLCT oil. (Personal Communication)

Nisshin (2006e) Monoglyceride & diglyceride content. (Personal Communication)

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Pages 000065-000077 removed under Freedom of Information exemption B4.

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January 19, 2007

Dr. Mary Ditto
U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Harvey W. Wiley Federal Building, HFS-255
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College Park, MD 20740-3835

Dear Dr. Ditto,

In response to our teleconference (January 8, 2007) concerning the removal of the designation of "business confidential" from the manufacturing section of the GRAS Notification for MLCT by Nisshin (2006), enclosed you should find the necessary replacement pages. Please redact page 6 of the GRAS Notification, as well as pages 9, 10, and 11 of the submitted GRAS dossier. Please append as supplemental, the enclosed pages 6, 9, 10, and 11, as appropriate. As requested, the designation of "business confidential" was removed from these pages, and the specific confidential terminology was altered to a more generic form.

If you have any questions, please let me know.

Sincerely,

(b)(6)

Ray A. Matulka, Ph.D.

(b)(6)

George A. Burdock, Ph.D., D.A.B.T.

000152

Table 3. Fatty acid composition of MLCT-Oil

Fatty Acid	M/L	*Percent* (%)*	Fatty Acid	M/L	*Percent* (%)
Caprylic acid (C8:0)	M	8.5-9.1	Linoleic acid (C18:2)	L	16.1-18.8
Capric acid (C10:0)	M	2.7-2.8	Linolenic acid (C18:3)	L	5.4-10.3
Lauric acid (C12:0)	L	ND	Arachidic acid (C20:0)	L	0.4-0.6
Myristic acid (C14:0)	L	ND	Gadoleic acid (C20:1)	L	0.9-1.2
Palmitic acid (C16:0)	L	3.2-4.0	Behenic acid (C22:0)	L	0.2-0.4
Palmitoleic acid (C16:1)	L	0.1-0.2	Erucic acid (C22:1)	L	0.1-0.3
Stearic acid (C18:0)	L	1.6-1.8	Lignoceric acid (C24:0)	L	0.1-0.2
Oleic acid (C18:1)	L	49.0-54.2	Nervonic acid (C24:1)	L	0.1-0.3

*As a *percent* of the total fatty acid content; M= medium-chain fatty acid; L= long-chain fatty acid; ND= not detected; MLCT= medium- and long-chain triacylglycerol

C. Method of Manufacture of Medium- and Long-chain Triacylglycerol Oil

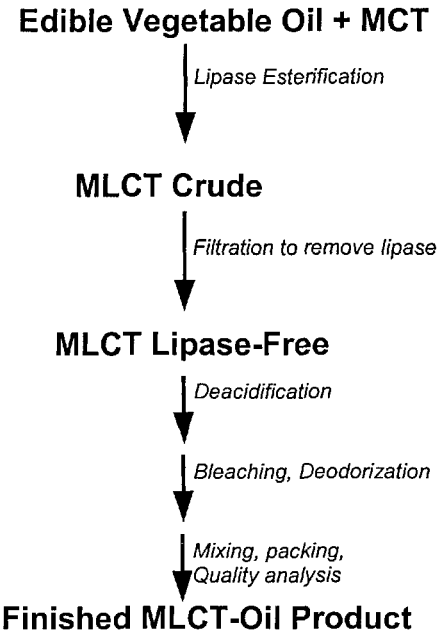

Figure 1. Medium- and long-chain triacylglycerol Oil (MLCT-Oil) production scheme

produced from coconut and palm kernel oils. Specific ratios of edible vegetable oil and MCT produced from edible oils are combined with a lipase utilized to promote a randomized ester exchange (Hoy and Xu, 2001) such that the MLCT-Oil produced is composed of both medium- and long-chain triacylglycerols (MLCT). The MCFA of MLCT-Oil consists only of caprylic and capric fatty acids. Since MLCT-Oil has been defined as including at least one LCFA molecule in a triacylglycerol, only a maximum of two MCFA will be included in the triacylglycerol molecule. The physical and chemical properties of MLCT-Oil are given in Table 3.

3.2. Manufacturing Process and Specifications of MLCT-Oil

3.2.1. Manufacture of MLCT-Oil

MLCT-Oil is manufactured in accordance with the manufacturing control standards and the quality control standards of the International Organization for Standardization (ISO) at the manufacturing facility of Nisshin Oillio Group, LTD. MLCT-Oil is manufactured from common edible vegetable oils (*e.g.*, rapeseed, soybean and cottonseed) and medium chain triacylglycerol (MCT) derived from edible coconut or palm kernel oil, by random transesterification using a bacterium-derived lipase (APPENDIX A). All of the constituents in MLCT-Oil are either approved food ingredients (GRAS), or are normal constituents found in commonly consumed foods at similar concentrations. The lipase has been deemed GRAS, and is derived from a source organism considered safe, as recommended by Pariza and Johnson (Pariza and Johnson, 2001) (APPENDIX A).

The two starting materials (*i.e.*, common vegetable oil and MCT) are produced by traditional manufacturing methods. For common vegetable oil (*e.g.,* rapeseed oil), manufacturing includes extraction, steam distillation, degumming, de-acidification (*i.e.*, alkali), bleaching, and deodorizing.[9] MCT is produced from coconut or palm kernel oil by saponification or hydrolysis[10] that produces mixed fatty acids. The fatty acid mixture is subjected to fractional distillation to isolate MCFA (*i.e.*, caprylic and capric acids). The MCFA are esterified with glycerin to produce a crude MCT product, which is purified using traditional oil processing procedures.

[9] Erickson, D.R. (1995) *Practical Handbook of Soybean Processing and Utilization.* AOCS Press and the United States Soybean Board, Champaign, IL and St. Louis, MO.

[10] High pressure steam

Enzymatic esterification is initiated by mixing vegetable oil with MCT at the appropriate temperature to produce a MLCT crude oil product (Figure 2). Esterification is initiated by allowing the vegetable oils (containing either LCFA or MCFA as raw materials) to be exposed to the lipase. The crude oil product is filtered to remove lipase (*i.e.*, lipase free MLCT). Then, the product is subjected to traditional oil processing (*i.e.*, de-acidification, bleaching, deodorizing, mixing, packing and analysis) to produce the final MLCT-Oil product. The MLCT-Oil product is washed with hot water during the de-acidification process, ensuring the complete removal of lipase from the product. To confirm that the lipase has been removed, acid production is measured, as residual lipase would contain hydrolytic activity and produce free fatty acids. Therefore, acid production is measured after agitation of a mixture of MLCT-Oil and water at the appropriate temperature, which facilitates any fatty acid production from residual lipase. The acid value after agitation is compared with the value prior to agitation. Significant increases in the acid value would warrant re-washing with hot water to remove any remaining lipase. The content of MCFA in MLCT-Oil is determined by the blending quantity of raw material used in the manufacturing process (Nisshin, 2006d). The manufacturer's quality assurance department will analyze the MLCT-Oil, and if the product does not meet specifications, the product will be reformulated to meet specifications presented in Table 3, or the MLCT-Oil batch will be discarded.

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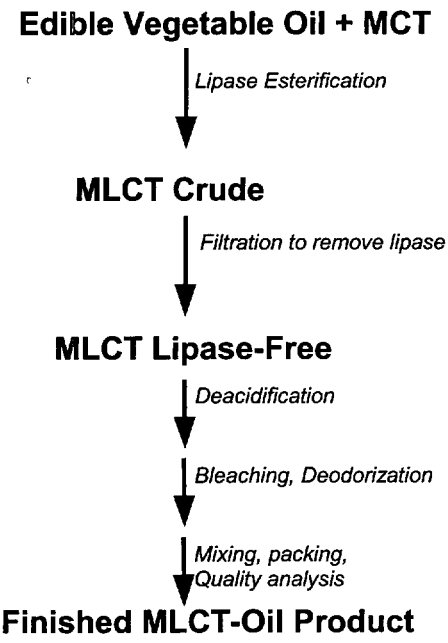

Figure 2. Detailed flow chart for the manufacture of MLCT-Oil (Nisshin, 2006d)

3.3. Summary of MLCT-Oil Identity and Manufacturing

The composition of MLCT-Oil is well characterized, thus its identity is well understood. It is composed of a glycerol with randomly bound medium and long chain fatty acids. The fatty acids are derived from common edible oils rich in free medium and long chain fatty acids. Compositional analysis indicates that the fatty acids present in MLCT-Oil are the type commonly found in other edible oils (Babayan, 1987; Kasai *et al.*, 2003)

The manufacturing process is important because not only must the final product be of suitable purity for consumption, but the materials used to produce MLCT-Oil must also be food grade. Commonly consumed vegetable oils (*e.g.*, rapeseed, soybean, cottonseed, and coconut or palm kernel oils) are utilized in the manufacture of MLCT-Oil. MLCT-Oil is manufactured by an esterification method. The oil contains approximately 12% MCFA, with the balance made up of LCFA (Table 2). MLCT-Oil specifications are provided in Table 3 (Nisshin, 2006a). As part of the specifications to identify MLCT-Oil, the fatty acid composition is presented in Table 1.

Ditto, Mary D

From: Ray Matulka [rmatulka@burdockgroup.com]
Sent: Friday, March 09, 2007 2:34 PM
To: Ditto, Mary D
Cc: George Burdock
Subject: RE: Questions regarding GRN 217

Attachments: Questions regarding GRN-217-Reply.pdf

Questions
garding GRN-217-Re

Dear Dr. Ditto,

As requested in your recent email (please see below), attached you should find a letter to amend the current GRAS dossier entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT" (GRAS Notification No. 000217). I will also send a hardcopy of the letter to your attention.

If you have any questions regarding these clarification amendments, or have trouble with the attachment, please let me know.

Sincerely,

Ray

Ray A. Matulka, Ph.D
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FAX: 772-562-3908

-----Original Message-----
From: Ditto, Mary D [mailto:mary.ditto@fda.hhs.gov]
Sent: Friday, February 23, 2007 1:37 PM
To: Ray Matulka
Subject: Questions regarding GRN 217

Dear Dr. Matulka,

Subsequent to our acknowledgment letter dated January 25, 2007, for GRN 000217, we are reconsidering the name of the substance that is the subject of the notice based on the fatty acid profile presented in the notice. At this point, we are planning to refer to the substance that is the subject of the notice as "tailored triglycerides containing approximately 12 percent medium chain fatty acids."

During our preliminary review of GRN 000217, we have identified the following items that require clarification. We would appreciate it if you could provide additional information about the following:

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In the GRAS Panel summary, the ingredient that is the subject of GRN 217 is described as containing approximately 75% long-chain fatty acids (as oleic, linoleic, and linolenic acids) (p. 45 of 64). However, in the description of the fatty acid composition (p. 7 of 64), the ingredient is described as containing 85.2% long-chain fatty acids. On page 5 of 64, in footnote 6, long chain fatty acids are defined as those that contain 16 carbons or more. This definition does not concur with the notations given in Table 1 (p. 7 of 64) or the parenthetical definition of LCFA given on p. 45. Please clarify the inconsistencies in the definition of LCFA and composition of LCFA in the tailored triglycerides ingredient.

In the GRAS Panel summary, Table 1 (p. 7 of 64), the fatty acid composition of the ingredient is provided. Please clarify the number of lots or batches analyzed to obtain these values.

Appendix A is marked "business confidential." Does the entire Appendix need to be confidential? If the "business confidential" label is removed from Appendix A then the following would not apply.
In the GRAS Panel summary, the discussion of the enzyme (p. 9 of 64) includes repeated references to Appendix A, which is stamped confidential. Please provide a non-CBI summary of the information in Appendix A that was reviewed by the GRAS Panel, including any information that the GRAS Panel relied on to conclude that the enzyme is safe and suitable for its intended use.

Please describe the conditions of use of the enzyme and state whether it is used in accordance with good manufacturing practices. Is the enzyme immobilized? Are all substances (e.g. processing aids) used in conjunction with the use of the enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

On p. 14 of the GRAS Panel summary, reference is made to Appendix B, which is stamped confidential. Please provide a non-CBI summary of the assumptions and relevant information associated with Nisshin OilliO's exposure estimate. For example, relevant information might include food categories, use levels, source of intake data, whether estimate represents eaters-only or the total population (eaters and non-eaters).

Please clarify if the intended uses include meat and poultry products. Although not listed in the "conditions of use" section of the GRAS notification, meat and poultry products are listed in the detailed exposure estimate in Appendix B stamped "business confidential".

There are varying descriptions in the text for how this oil will be used (e.g., as supplementary source of vegetable oil used in cooking, as an ingredient in specific foods- salad dressings, margarine spreads, and frozen dinners; as a replacement for vegetable oil). It is our understanding that the intended use is as a replacement for vegetable oil for use in food in general. Is this correct?

Please provide a specification for lead (rather than a specification for heavy metals as lead).

Sometimes we receive microbial specifications. Do you have such specifications for this ingredient?

We expect that the information we have requested is readily available to you and should not require substantial data collection and preparation. If you choose to respond by post we would appreciate a copy of the response either by email (mary.ditto@fda.hhs.gov) or fax (301-436-2964) so that we may proceed with the evaluation of your notice as soon as possible. If you have any questions about our request, or if you believe you will not be able to respond within approximately two weeks, please feel free to contact me either by email or by phone during FDA business hours at 301-436-1165.

Sincerely,

000164

Mary Ditto, Ph.D.
Office of Food Additive Safety
Division of Biotechnology
& GRAS Notice Review
Telephone: 301-436-1165
Telefax: 301-436-2965

New email address: mary.ditto@fda.hhs.gov

000165

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March 9, 2007

Mary Ditto, Ph.D.
Office of Food Additive Safety
Division of Biotechnology & GRAS Notice Review
Telephone: 301-436-1165
Telefax: 301-436-2965

New email address: mary.ditto@fda.hhs.gov

RE: Questions regarding GRN-217

Dear Dr. Ditto:

This letter is to amend the current GRAS dossier[1] entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT," which requests the use of MLCT-Oil as a replacement of common vegetable oil used in cooking, salad dressings, margarine spreads, and frozen dinners. Several points have been raised concerning this GRAS dossier, and based on the email from you dated February 23, 2007 (please see APPENDIX I), and we submit the following clarification amendments to the pending GRAS Notification (GRN Notice No. 000217).

This clarification addresses several issues that were presented by the FDA, with the following responses:

1. The FDA would like to refer to the substance that is the subject of the notice as "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids."

[1] GRAS Notification Letter dated December 14, 2006; GRN No. 000217.

000166

We have no objection to the designation "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids."[2]

2. In the GRAS Panel summary, the ingredient that is the subject of GRN 217 is described as containing approximately 75% long-chain fatty acids (as oleic, linoleic, and linolenic acids) (p. 45 of 64). However, in the description of the fatty acid composition (p. 7 of 64), the ingredient is described as containing 85.2% long-chain fatty acids. On page 5 of 64, in footnote 6, long chain fatty acids are defined as those that contain 16 carbons or more. This definition does not concur with the notations given in Table 1 (p. 7 of 64) or the parenthetical definition of LCFA given on p. 45. Please clarify the inconsistencies in the definition of LCFA and composition of LCFA in the tailored triglycerides ingredient.

To clarify, the tailored triglyceride oil contains approximately 85.2% long-chain fatty acids (based on the total amount of fatty acids present in the tailored triglyceride oil). Oleic, linoleic, and linolenic fatty acids represent a significant portion of the long-chain fatty acids (approximately 88% of the total long-chain fatty acids present, or 75% of the total fatty acids present in the tailored triglyceride oil). As the literature is inconsistent with the definition of "medium" or "long" to denote chain length of lauric and myristic acid, please disregard the "M" or "L" notation in Table 1 of the GRAS Notification No. 000217. In the context of this GRAS dossier, long-chain fatty acids are defined as fatty acids with a chain length of 16 carbons or greater.

3. In the GRAS Panel summary, Table 1 (p. 7 of 64), the fatty acid composition of the ingredient is provided. Please clarify the number of lots or batches analyzed to obtain these values.

The fatty acid compositional data provided in Table 1 of GRAS Notification No. 000217 was provided from five lots, no more than three of which were consecutive.

4. Appendix A is marked "business confidential." Does the entire Appendix need to be confidential? If the "business confidential" label is removed from Appendix A then the following would not apply.
In the GRAS Panel summary, the discussion of the enzyme (p. 9 of 64) includes repeated references to Appendix A, which is stamped confidential. Please provide a non-CBI summary of the information in Appendix A that was reviewed by the GRAS Panel, including any information that the GRAS Panel relied on to conclude that the enzyme is safe and suitable for its intended use.

The label "business confidential" may be removed from Appendix A of the GRAS Notification No. 000217.

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[2] Synonyms for "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids" include "MLCT-Oil", and "tailored triglyceride oil".

5. Please describe the conditions of use of the enzyme and state whether it is used in accordance with good manufacturing practices. Is the enzyme immobilized? Are all substances (e.g. processing aids) used in conjunction with the use of the enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

The enzyme is immobilized, with all substances used in conjunction with the enzyme safe and in compliance with all applicable legal and regulatory requirements. As indicated in the attached letter (APPENDIX II), use of the enzyme in the manufacture of the tailored triglyceride oil is conducted under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry and Fisheries (MAFF) procedures, which are consistent with good manufacturing practices.

6. On p. 14 of the GRAS Panel summary, reference is made to Appendix B, which is stamped confidential. Please provide a non-CBI summary of the assumptions and relevant information associated with Nisshin OilliO's exposure estimate. For example, relevant information might include food categories, use levels, source of intake data, whether estimate represents eaters-only or the total population (eaters and non-eaters).

In order to determine a hypothetical maximum daily ingestion of the tailored triglyceride oil as an added ingredient by humans, a consumption analysis database[3] was analyzed for consumption of the tailored triglyceride oil when added to specific foods (as a replacement for cooking oil, and when added to salad dressings, margarine spreads, and frozen dinners, as indicated in Appendix B of the GRAS Notification No. 000217). This nationwide dietary intake survey was conducted during 2001-2002, and was comprised of two days of data that was collected for all respondents in the food survey (n = 9,701 individuals). Food categories for the addition of the tailored triglyceride oil were designated by Nisshin OilliO Group, Ltd. The addition of the tailored triglyceride oil to a food for which a standard of identity exists would demand that the food product be named other than that as indicated under the standard of identity.

The maximum concentration of the tailored triglyceride oil suggested was utilized in the consumption analysis, to ensure that the estimate includes any individuals that consume larger amounts of the foods. The concentrations of the tailored triglyceride oil for the specific food codes were calculated from the raw ingredient data included in the standard recipe files from the United States Department of Agriculture (USDA) 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII), and converted to mg/g concentrations.[4] Although the ingredient may be added to only one part of a complex food mixture (*i.e.*, "beef with barbeque sauce"), the consumption of the total food was utilized in the consumption analysis calculations. The results were weighted to place more strength on foods that were consumed by more individuals, and extrapolated to the US population. The consumption of the tailored triglyceride oil was then determined for eaters only, based on the consumption of the specific foods.

---

[3] Source: HHS What We Eat in America, National Health and Nutrition Examination Survey (NHANES) 2001-2002, USDA

[4] Source: CSFII 1994-96 (2000) *Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96, 98.* Agricultural Research Service, US Department of Agriculture, Washington, DC. CD-ROM.

The tailored triglyceride oil use levels are presented in Appendix B of the GRAS Notification No. 000217 (please remove the "business confidential" designation for this appendix).

7. Please clarify if the intended uses include meat and poultry products. Although not listed in the "conditions of use" section of the GRAS notification, meat and poultry products are listed in the detailed exposure estimate in Appendix B stamped "business confidential".

The frozen dinner category includes meat and poultry products, indicated in Appendix B of the GRAS Notification No. 000217. As indicated under the Memorandum of Understanding between FDA and USDA, please inform the USDA of this use.

8. There are varying descriptions in the text for how this oil will be used (e.g., as supplementary source of vegetable oil used in cooking, as an ingredient in specific foods-salad dressings, margarine spreads, and frozen dinners; as a replacement for vegetable oil). It is our understanding that the intended use is as a replacement for vegetable oil for use in food in general. Is this correct?

The tailored triglyceride oil is to be used in cooking, salad dressings, margarine spreads, and frozen dinners, as indicated in APPENDIX B of the GRAS Notification No. 000217. The tailored triglyceride oil is not intended as a replacement for all vegetable oil use in food in general, but only in those named categories.

9. Please provide a specification for lead (rather than a specification for heavy metals as lead).

The specification for lead has been determined as a lead concentration of not more than 0.1 mg/kg, as indicated in the attached letter (APPENDIX III).

10. Sometimes we receive microbial specifications. Do you have such specifications for this ingredient?

Specifications for the tailored triglyceride oil meet those stated in the Food Chemicals Codex for similar oils, which do not include microbial specifications. We feel that the inclusion of microbial specifications for the tailored triglyceride oil is not necessary for this GRAS Notification No. 000217.

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Summary

In summary, the designation of the tailored triglyceride oil as "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids" is appropriate for GRN No. 000217. Long-chain fatty acids contained in the tailored triglyceride oil consist of fatty acids with chain lengths of 16 carbons or greater, and comprises approximately 85.2% of the total amount of fatty acids present in the tailored triglyceride oil. This fatty acid compositional data was provided from five lots of the tailored triglyceride oil, no more than three of which were consecutive, produced with an immobilized enzyme and other safe ingredients, in compliance with all applicable legal and regulatory requirements, according to Japanese standards consistent with good manufacturing practices. The exposure of the tailored triglyceride oil was determined for eaters only, calculated from data available from a nationwide dietary intake survey and concentrations in the specific foods from USDA standard recipe files. The tailored triglyceride oil is to be used in the replacement of cooking oil, and in salad dressings, margarine spreads, and frozen dinners, in which the frozen dinners may contain meat and poultry products; therefore the USDA must be informed of this use. The tailored triglyceride oil is not to be used as a replacement for vegetable oil in general and for all foods, but as a replacement for vegetable oil only for the specific foods identified herein. The specific specification for lead for the tailored triglyceride oil is no more than 0.1 mg/kg, while microbial specifications for the tailored triglyceride oil and similar vegetable oils (as indicated in the Food Chemicals Codex) are not warranted.

If you have any further questions regarding these or any other topics concerning the GRAS Notification of the tailored triglyceride oil, containing approximately 12 *percent* medium chain fatty acids, to be used in cooking, salad dressings, margarine spreads, and frozen dinners, please contact me.

Sincerely,

(b)(6)

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

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APPENDIX I

-----Original Message-----
From: Ditto, Mary D [mailto:mary.ditto@fda.hhs.gov]
Sent: Friday, February 23, 2007 1:37 PM
To: Ray Matulka
Subject: Questions regarding GRN 217

Dear Dr. Matulka,

Subsequent to our acknowledgment letter dated January 25, 2007, for GRN 000217, we are reconsidering the name of the substance that is the subject of the notice based on the fatty acid profile presented in the notice. At this point, we are planning to refer to the substance that is the subject of the notice as "tailored triglycerides containing approximately 12 percent medium chain fatty acids."

During our preliminary review of GRN 000217, we have identified the following items that require clarification. We would appreciate it if you could provide additional information about the following:

In the GRAS Panel summary, the ingredient that is the subject of GRN 217 is described as containing approximately 75% long-chain fatty acids (as oleic, linoleic, and linolenic acids) (p. 45 of 64). However, in the description of the fatty acid composition (p. 7 of 64), the ingredient is described as containing 85.2% long-chain fatty acids. On page 5 of 64, in footnote 6, long chain fatty acids are defined as those that contain 16 carbons or more. This definition does not concur with the notations given in Table 1 (p. 7 of 64) or the parenthetical definition of LCFA given on p. 45. Please clarify the inconsistencies in the definition of LCFA and composition of LCFA in the tailored triglycerides ingredient.

In the GRAS Panel summary, Table 1 (p. 7 of 64), the fatty acid composition of the ingredient is provided. Please clarify the number of lots or batches analyzed to obtain these values.

Appendix A is marked "business confidential." Does the entire Appendix need to be confidential? If the "business confidential" label is removed from Appendix A then the following would not apply.
In the GRAS Panel summary, the discussion of the enzyme (p. 9 of 64) includes repeated references to Appendix A, which is stamped confidential. Please provide a non-CBI summary of the information in Appendix A that was reviewed by the GRAS Panel, including any information that the GRAS Panel relied on to conclude that the enzyme is safe and suitable for its intended use.

Please describe the conditions of use of the enzyme and state whether it is used in accordance with good manufacturing practices. Is the enzyme immobilized? Are all substances (e.g. processing aids) used in conjunction with the use of the enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

On p. 14 of the GRAS Panel summary, reference is made to Appendix B, which is stamped confidential. Please provide a non-CBI summary of the assumptions

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and relevant information associated with Nisshin OilliO's exposure estimate. For example, relevant information might include food categories, use levels, source of intake data, whether estimate represents eaters-only or the total population (eaters and non-eaters).

Please clarify if the intended uses include meat and poultry products. Although not listed in the "conditions of use" section of the GRAS notification, meat and poultry products are listed in the detailed exposure estimate in Appendix B stamped "business confidential".

There are varying descriptions in the text for how this oil will be used (e.g., as supplementary source of vegetable oil used in cooking, as an ingredient in specific foods- salad dressings, margarine spreads, and frozen dinners; as a replacement for vegetable oil). It is our understanding that the intended use is as a replacement for vegetable oil for use in food in general. Is this correct?

Please provide a specification for lead (rather than a specification for heavy metals as lead).

Sometimes we receive microbial specifications. Do you have such specifications for this ingredient?

We expect that the information we have requested is readily available to you and should not require substantial data collection and preparation. If you choose to respond by post we would appreciate a copy of the response either by email (mary.ditto@fda.hhs.gov) or fax (301-436-2964) so that we may proceed with the evaluation of your notice as soon as possible. If you have any questions about our request, or if you believe you will not be able to respond within approximately two weeks, please feel free to contact me either by email or by phone during FDA business hours at 301-436-1165.

Sincerely,

Mary Ditto, Ph.D.
Office of Food Additive Safety
Division of Biotechnology
& GRAS Notice Review
Telephone: 301-436-1165
Telefax: 301-436-2965

New email address: mary.ditto@fda.hhs.gov

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APPENDIX II

The following letter indicates the manufacture of MLCT-Oil under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry and Fisheries (MAFF) regulations from OilliO-USA (please see attached).

March 9, 2007

To Whom It May Concern:

We hereby confirm that the manufacture of MLCT-Oil conforms to the regulations under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry, and Fisheries (MAFF), which are consistent with providing food quality under current Good Manufacturing Practices (GMP) to help ensure that MLCT-Oil is consistently a safe, high quality product.

Sincerely,

(b)(6)

Katsuaki Yamanouchi
Co-Vice President
The Nisshin OilliO Group USA, Inc.
120 Charlotte Place
Mid Level
Englewood Cliffs, NJ 07632
Phone: 201-871-4020
Fax: 201-871-4090

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APPENDIX III

The following letter describes the lead specification for the tailored triglyceride oil defined in GRAS Notification No. 000217 (please see attached).

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NISSHIN OilliO

March 9, 2007

To Whom It May Concern:

This is to certify that based on analytical chemistry results utilizing Atomic Absorption Spectrometry, conducted according to Japan Food Research Laboratories's Standard Operating Procedure (available on request), MLCT-Oil conforms to the lead specification:

Lead concentration: not more than 0.1 mg/kg

Sincerely,

(b)(6)

Katsuaki Yamanouchi
Co-Vice President
The Nisshin OilliO Group USA, Inc.
120 Charlotte Place
Mid Level
Englewood Cliffs, NJ 07632
Phone: 201-871-4020
Fax: 201-871-4090

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AM

March 12, 2007

Mary Ditto, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Division of Biotechnology & GRAS Notice Review
Food and Drug Administration
5100 Pain Branch Parkway,
College Park, MD 20740
Tel: 301-436-1165

Dear Dr. Ditto,

As indicated in a recent email (09MAR07), enclosed you should find a hard copy of the GRAS Amendment for GRN 000217 entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT". A pdf of this GRAS Amendment was emailed to you on 09MAR07.

If you have any questions, please let me know.

Sincerely,

(b)(6)

George A. Burdock, Ph.D.

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March 9, 2007

Mary Ditto, Ph.D.
Office of Food Additive Safety
Division of Biotechnology & GRAS Notice Review
Telephone: 301-436-1165
Telefax: 301-436-2965

New email address: mary.ditto@fda.hhs.gov

RE: Questions regarding GRN-217

Dear Dr. Ditto:

This letter is to amend the current GRAS dossier[1] entitled "DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MEDIUM- AND LONG-CHAIN TRIACYLGLYCEROL (MLCT)-OIL AS A FOOD INGREDIENT," which requests the use of MLCT-Oil as a replacement of common vegetable oil used in cooking, salad dressings, margarine spreads, and frozen dinners. Several points have been raised concerning this GRAS dossier, and based on the email from you dated February 23, 2007 (please see APPENDIX I), and we submit the following clarification amendments to the pending GRAS Notification (GRN Notice No. 000217).

This clarification addresses several issues that were presented by the FDA, with the following responses:

1. The FDA would like to refer to the substance that is the subject of the notice as "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids."

[1] GRAS Notification Letter dated December 14, 2006; GRN No. 000217.

000179

We have no objection to the designation "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids."[2]

2. In the GRAS Panel summary, the ingredient that is the subject of GRN 217 is described as containing approximately 75% long-chain fatty acids (as oleic, linoleic, and linolenic acids) (p. 45 of 64). However, in the description of the fatty acid composition (p. 7 of 64), the ingredient is described as containing 85.2% long-chain fatty acids. On page 5 of 64, in footnote 6, long chain fatty acids are defined as those that contain 16 carbons or more. This definition does not concur with the notations given in Table 1 (p. 7 of 64) or the parenthetical definition of LCFA given on p. 45. Please clarify the inconsistencies in the definition of LCFA and composition of LCFA in the tailored triglycerides ingredient.

To clarify, the tailored triglyceride oil contains approximately 85.2% long-chain fatty acids (based on the total amount of fatty acids present in the tailored triglyceride oil). Oleic, linoleic, and linolenic fatty acids represent a significant portion of the long-chain fatty acids (approximately 88% of the total long-chain fatty acids present, or 75% of the total fatty acids present in the tailored triglyceride oil). As the literature is inconsistent with the definition of "medium" or "long" to denote chain length of lauric and myristic acid, please disregard the "M" or "L" notation in Table 1 of the GRAS Notification No. 000217. In the context of this GRAS dossier, long-chain fatty acids are defined as fatty acids with a chain length of 16 carbons or greater.

3. In the GRAS Panel summary, Table 1 (p. 7 of 64), the fatty acid composition of the ingredient is provided. Please clarify the number of lots or batches analyzed to obtain these values.

The fatty acid compositional data provided in Table 1 of GRAS Notification No. 000217 was provided from five lots, no more than three of which were consecutive.

4. Appendix A is marked "business confidential." Does the entire Appendix need to be confidential? If the "business confidential" label is removed from Appendix A then the following would not apply.
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The label "business confidential" may be removed from Appendix A of the GRAS Notification No. 000217.

---

[2] Synonyms for "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids" include "MLCT-Oil", and "tailored triglyceride oil".

5. Please describe the conditions of use of the enzyme and state whether it is used in accordance with good manufacturing practices. Is the enzyme immobilized? Are all substances (e.g. processing aids) used in conjunction with the use of the enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

The enzyme is immobilized, with all substances used in conjunction with the enzyme safe and in compliance with all applicable legal and regulatory requirements. As indicated in the attached letter (APPENDIX II), use of the enzyme in the manufacture of the tailored triglyceride oil is conducted under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry and Fisheries (MAFF) procedures, which are consistent with good manufacturing practices.

6. On p. 14 of the GRAS Panel summary, reference is made to Appendix B, which is stamped confidential. Please provide a non-CBI summary of the assumptions and relevant information associated with Nisshin OilliO's exposure estimate. For example, relevant information might include food categories, use levels, source of intake data, whether estimate represents eaters-only or the total population (eaters and non-eaters).

In order to determine a hypothetical maximum daily ingestion of the tailored triglyceride oil as an added ingredient by humans, a consumption analysis database[3] was analyzed for consumption of the tailored triglyceride oil when added to specific foods (as a replacement for cooking oil, and when added to salad dressings, margarine spreads, and frozen dinners, as indicated in Appendix B of the GRAS Notification No. 000217). This nationwide dietary intake survey was conducted during 2001-2002, and was comprised of two days of data that was collected for all respondents in the food survey (n = 9,701 individuals). Food categories for the addition of the tailored triglyceride oil were designated by Nisshin OilliO Group, Ltd. The addition of the tailored triglyceride oil to a food for which a standard of identity exists would demand that the food product be named other than that as indicated under the standard of identity.

The maximum concentration of the tailored triglyceride oil suggested was utilized in the consumption analysis, to ensure that the estimate includes any individuals that consume larger amounts of the foods. The concentrations of the tailored triglyceride oil for the specific food codes were calculated from the raw ingredient data included in the standard recipe files from the United States Department of Agriculture (USDA) 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (CSFII), and converted to mg/g concentrations.[4] Although the ingredient may be added to only one part of a complex food mixture (*i.e.*, "beef with barbeque sauce"), the consumption of the total food was utilized in the consumption analysis calculations. The results were weighted to place more strength on foods that were consumed by more individuals, and extrapolated to the US population. The consumption of the tailored triglyceride oil was then determined for eaters only, based on the consumption of the specific foods.

---

[3] Source: HHS What We Eat in America, National Health and Nutrition Examination Survey (NHANES) 2001-2002, USDA

[4] Source: CSFII 1994-96 (2000) *Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96, 98.* Agricultural Research Service, US Department of Agriculture, Washington, DC. CD-ROM.

The tailored triglyceride oil use levels are presented in Appendix B of the GRAS Notification No. 000217 (please remove the "business confidential" designation for this appendix).

7. Please clarify if the intended uses include meat and poultry products. Although not listed in the "conditions of use" section of the GRAS notification, meat and poultry products are listed in the detailed exposure estimate in Appendix B stamped "business confidential".

The frozen dinner category includes meat and poultry products, indicated in Appendix B of the GRAS Notification No. 000217. As indicated under the Memorandum of Understanding between FDA and USDA, please inform the USDA of this use.

8. There are varying descriptions in the text for how this oil will be used (e.g., as supplementary source of vegetable oil used in cooking, as an ingredient in specific foods-salad dressings, margarine spreads, and frozen dinners; as a replacement for vegetable oil). It is our understanding that the intended use is as a replacement for vegetable oil for use in food in general. Is this correct?

The tailored triglyceride oil is to be used in cooking, salad dressings, margarine spreads, and frozen dinners, as indicated in APPENDIX B of the GRAS Notification No. 000217. The tailored triglyceride oil is not intended as a replacement for all vegetable oil use in food in general, but only in those named categories.

9. Please provide a specification for lead (rather than a specification for heavy metals as lead).

The specification for lead has been determined as a lead concentration of not more than 0.1 mg/kg, as indicated in the attached letter (APPENDIX III).

10. Sometimes we receive microbial specifications. Do you have such specifications for this ingredient?

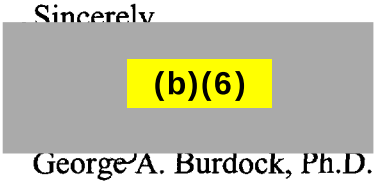
Specifications for the tailored triglyceride oil meet those stated in the Food Chemicals Codex for similar oils, which do not include microbial specifications. We feel that the inclusion of microbial specifications for the tailored triglyceride oil is not necessary for this GRAS Notification No. 000217.

Summary

In summary, the designation of the tailored triglyceride oil as "tailored triglycerides containing approximately 12 *percent* medium chain fatty acids" is appropriate for GRN No. 000217. Long-chain fatty acids contained in the tailored triglyceride oil consist of fatty acids with chain lengths of 16 carbons or greater, and comprises approximately 85.2% of the total amount of fatty acids present in the tailored triglyceride oil. This fatty acid compositional data was provided from five lots of the tailored triglyceride oil, no more than three of which were consecutive, produced with an immobilized enzyme and other safe ingredients, in compliance with all applicable legal and regulatory requirements, according to Japanese standards consistent with good manufacturing practices. The exposure of the tailored triglyceride oil was determined for eaters only, calculated from data available from a nationwide dietary intake survey and concentrations in the specific foods from USDA standard recipe files. The tailored triglyceride oil is to be used in the replacement of cooking oil, and in salad dressings, margarine spreads, and frozen dinners, in which the frozen dinners may contain meat and poultry products; therefore the USDA must be informed of this use. The tailored triglyceride oil is not to be used as a replacement for vegetable oil in general and for all foods, but as a replacement for vegetable oil only for the specific foods identified herein. The specific specification for lead for the tailored triglyceride oil is no more than 0.1 mg/kg, while microbial specifications for the tailored triglyceride oil and similar vegetable oils (as indicated in the Food Chemicals Codex) are not warranted.

If you have any further questions regarding these or any other topics concerning the GRAS Notification of the tailored triglyceride oil, containing approximately 12 *percent* medium chain fatty acids, to be used in cooking, salad dressings, margarine spreads, and frozen dinners, please contact me.

Sincerely



George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

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APPENDIX I

-----Original Message-----
From: Ditto, Mary D [mailto:mary.ditto@fda.hhs.gov]
Sent: Friday, February 23, 2007 1:37 PM
To: Ray Matulka
Subject: Questions regarding GRN 217

Dear Dr. Matulka,

Subsequent to our acknowledgment letter dated January 25, 2007, for GRN 000217, we are reconsidering the name of the substance that is the subject of the notice based on the fatty acid profile presented in the notice. At this point, we are planning to refer to the substance that is the subject of the notice as "tailored triglycerides containing approximately 12 percent medium chain fatty acids."

During our preliminary review of GRN 000217, we have identified the following items that require clarification. We would appreciate it if you could provide additional information about the following:

In the GRAS Panel summary, the ingredient that is the subject of GRN 217 is described as containing approximately 75% long-chain fatty acids (as oleic, linoleic, and linolenic acids) (p. 45 of 64). However, in the description of the fatty acid composition (p. 7 of 64), the ingredient is described as containing 85.2% long-chain fatty acids. On page 5 of 64, in footnote 6, long chain fatty acids are defined as those that contain 16 carbons or more. This definition does not concur with the notations given in Table 1 (p. 7 of 64) or the parenthetical definition of LCFA given on p. 45. Please clarify the inconsistencies in the definition of LCFA and composition of LCFA in the tailored triglycerides ingredient.

In the GRAS Panel summary, Table 1 (p. 7 of 64), the fatty acid composition of the ingredient is provided. Please clarify the number of lots or batches analyzed to obtain these values.

Appendix A is marked "business confidential." Does the entire Appendix need to be confidential? If the "business confidential" label is removed from Appendix A then the following would not apply.
In the GRAS Panel summary, the discussion of the enzyme (p. 9 of 64) includes repeated references to Appendix A, which is stamped confidential. Please provide a non-CBI summary of the information in Appendix A that was reviewed by the GRAS Panel, including any information that the GRAS Panel relied on to conclude that the enzyme is safe and suitable for its intended use.

Please describe the conditions of use of the enzyme and state whether it is used in accordance with good manufacturing practices. Is the enzyme immobilized? Are all substances (e.g. processing aids) used in conjunction with the use of the enzyme safe and otherwise in compliance with all applicable legal and regulatory requirements?

On p. 14 of the GRAS Panel summary, reference is made to Appendix B, which is stamped confidential. Please provide a non-CBI summary of the assumptions

and relevant information associated with Nisshin OilliO's exposure estimate. For example, relevant information might include food categories, use levels, source of intake data, whether estimate represents eaters-only or the total population (eaters and non-eaters).

Please clarify if the intended uses include meat and poultry products. Although not listed in the "conditions of use" section of the GRAS notification, meat and poultry products are listed in the detailed exposure estimate in Appendix B stamped "business confidential".

There are varying descriptions in the text for how this oil will be used (e.g., as supplementary source of vegetable oil used in cooking, as an ingredient in specific foods- salad dressings, margarine spreads, and frozen dinners; as a replacement for vegetable oil). It is our understanding that the intended use is as a replacement for vegetable oil for use in food in general. Is this correct?

Please provide a specification for lead (rather than a specification for heavy metals as lead).

Sometimes we receive microbial specifications. Do you have such specifications for this ingredient?

We expect that the information we have requested is readily available to you and should not require substantial data collection and preparation. If you choose to respond by post we would appreciate a copy of the response either by email (mary.ditto@fda.hhs.gov) or fax (301-436-2964) so that we may proceed with the evaluation of your notice as soon as possible. If you have any questions about our request, or if you believe you will not be able to respond within approximately two weeks, please feel free to contact me either by email or by phone during FDA business hours at 301-436-1165.

Sincerely,

Mary Ditto, Ph.D.
Office of Food Additive Safety
Division of Biotechnology
& GRAS Notice Review
Telephone: 301-436-1165
Telefax: 301-436-2965

New email address: mary.ditto@fda.hhs.gov

APPENDIX II

The following letter indicates the manufacture of MLCT-Oil under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry and Fisheries (MAFF) regulations from OilliO-USA (please see attached).

NISSHIN OilliO THE NISSHIN OILLIO GROUP USA, INC.

March 9, 2007

To Whom It May Concern:

We hereby confirm that the manufacture of MLCT-Oil conforms to the regulations under Japan's Department of Food Safety, Ministry of Health, Labor and Welfare (MHLW), and the Ministry of Agriculture, Forestry, and Fisheries (MAFF), which are consistent with providing food quality under current Good Manufacturing Practices (GMP) to help ensure that MLCT-Oil is consistently a safe, high quality product.

Sincerely,

(b)(6)

Katsuaki Yamanouchi
Co-Vice President
The Nisshin OilliO Group USA, Inc.
120 Charlotte Place
Mid Level
Englewood Cliffs, NJ 07632
Phone: 201-871-4020
Fax: 201-871-4090

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APPENDIX III

The following letter describes the lead specification for the tailored triglyceride oil defined in GRAS Notification No. 000217 (please see attached).

NISSHIN OilliO THE NISSHIN OILLIO GROUP USA, INC.

March 9, 2007

To Whom It May Concern:

This is to certify that based on analytical chemistry results utilizing Atomic Absorption Spectrometry, conducted according to Japan Food Research Laboratories's Standard Operating Procedure (available on request), MLCT-Oil conforms to the lead specification:

Lead concentration: not more than 0.1 mg/kg

Sincerely,

(b)(6)

Katsuaki Yamanouchi
Co-Vice President
The Nisshin OilliO Group USA, Inc.
120 Charlotte Place
Mid Level
Englewood Cliffs, NJ 07632
Phone: 201-871-4020
Fax: 201-871-4090

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